Intranasal ST266 Mitigates Chronic Neuroinflammation and White Matter Pathology Following Repetitive Head Injury

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Introduction

Traumatic brain injury (TBI) is a prevalent and serious health issue among Veterans, whether sustained in combat or during training exercises. Current care and assessment standards remain inadequate. To address the long-term effects of repetitive mild TBI (r-mTBI), we are evaluating the therapeutic potential of intranasal **ST266** in an established r-mTBI animal model.

ST266 is a novel, cell-free biologic derived from secretions of full-term human placental cells. **It contains anti-inflammatory and neuroprotective factors** and is delivered intranasally to the upper nasal cavity near the brain (cribriform plate) for targeted therapeutic effect. This project aims to better understand the chronic effects of TBI, with or without **ST266** treatment. We hypothesize that delayed and sustained intranasal **ST266** administration will reduce the functional and neuropathological consequences of r-mTBI.

Emerging research suggests that TBI can alter the gut microbiome, likely through bidirectional interactions within the gut-brain axis. To explore this connection, we collected fecal samples before treatment to assess baseline microbiome changes caused by TBI. Post-treatment samples will help determine whether **ST266** can help restore a healthy gut environment. This is especially relevant as the same **ST266** secretome is also being investigated by Noveome for treating necrotizing enterocolitis (NEC) in premature infants. Our analysis examines microbiome shifts from broad (phylum) to specific (species) bacterial levels.

ST266 is a multi-targeted, non-cellular platform biologic produced by **Noveome Biotherapeutics** (Pittsburgh, PA) with the potential to improve patients' outcomes across a range of diseases and conditions in ophthalmology, neurology and more.

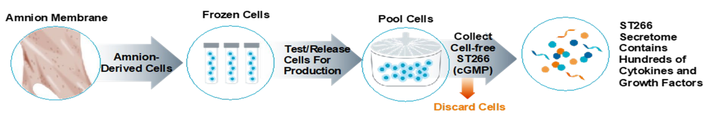
Neuroprotection
ST266 restores nerve function, supports nerve cell survival and decreases the rate of demyelination.

Anti-inflammation
ST266 modulates the inflammatory response by decreasing pro-inflammatory cytokines and reducing vascular permeability, immune cell infiltration and edema.

Restoration
ST266 improves function and recovery of damaged cells, increases cell proliferation, reduces apoptosis and helps re-establish homeostasis.

Healing
ST266 supports normal tissue repair by promoting collagen fiber deposition, alignment, and cross-linking. It may also accelerate healing in impaired conditions, including those associated with aging or diabetes.

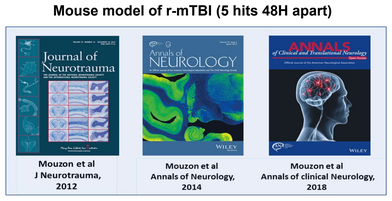
ST266 Production Flowchart



In August 2024, a clinical trial began testing ST266 in premature infants with necrotizing enterocolitis, a serious condition causing intestinal tissue death. The study aims to evaluate the safety, tolerability, and effectiveness of ST266 in this vulnerable population.

Methodology

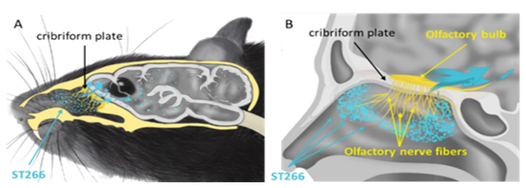
Mouse model of r-mTBI (5 hits 48H apart)



Mouzon et al J Neurotrauma, 2012 | Mouzon et al Annals of Neurology, 2014 | Mouzon et al Annals of clinical Neurology, 2018

A well-established animal model of repetitive closed head injury that mimics clinically relevant symptoms to advance research on mild TBI/concussion.

This study includes **160** C57BL/6J mice (2 sexes), divided by exposure (Sham or r-mTBI) and treatment (ST266 or vehicle), with daily intranasal dosing that will start at 18 months post-injury and continuing for 4 months. The mice are currently aging to reach the required post-injury timepoint. Anxiety-like behavior was assessed at 24 hours and 6 months post-injury. Fecal samples were collected at 6 months, and blood was collected at 24 hours after the last injury.



Nose to brain route of administration of ST266 to the central nervous system in a mouse **(A)** and in human **(B)** bypassing the blood-brain barrier in the process

Outline of experimental schedule

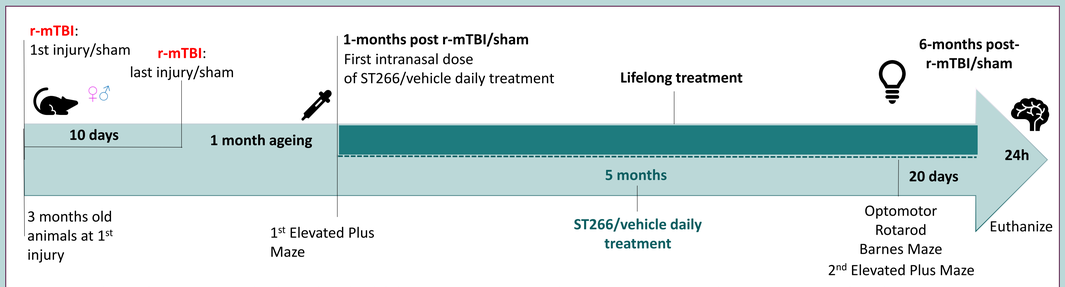


Fig 1. Outline of experimental schedule. The lifelong treatment paradigm starts at 1 month post injury until euthanasia.

Pre-clinical safety evaluation and biodistribution studies in rats and cynomolgus monkeys reported that I-125 labeled ST266 bypasses the blood-brain barrier (BBB) via the olfactory nerves and cribriform plate.

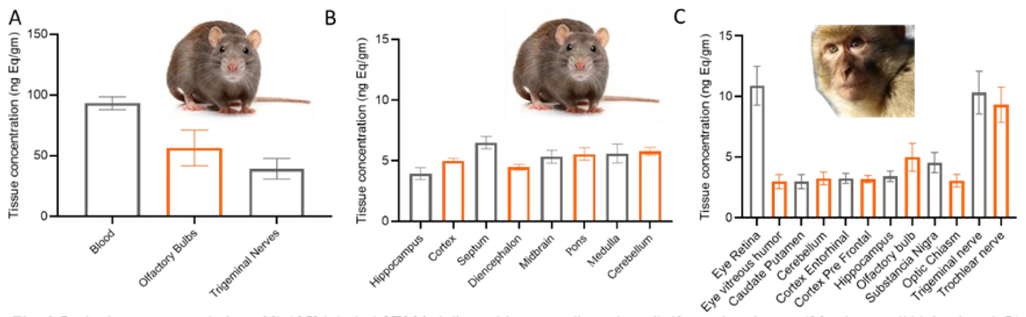
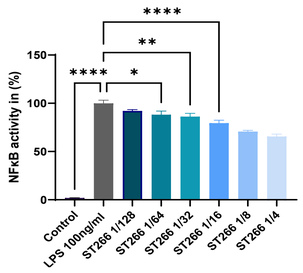


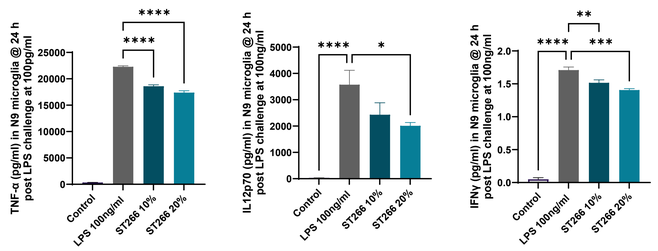
Fig. 2 Brain tissue accumulation of [I-125]-labeled ST266 delivered intranasally to the cribriform plate in rats (30 min post IN injection; A,B) and in cynomolgus monkeys (1 h post IN injection; C). Each bar represent the mean of 4 animals for the rats and 3 animals for the monkeys.

ST266 Reduces TNF-α–Induced NF-κB Activation in a Dose-Dependent Manner



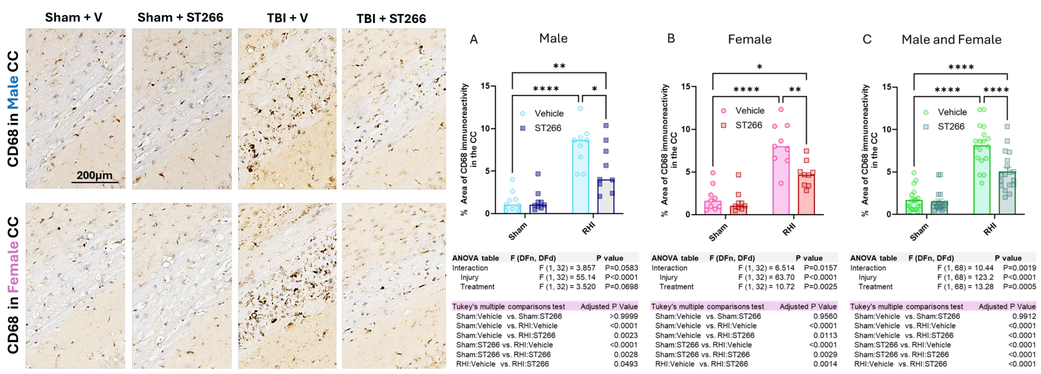
ST266 dose-dependently suppresses TNF-α–induced NF-κB activation in a HEK293 reporter cell line. NF-κB-luciferase reporter HEK293 cells were stimulated with TNF-α (25 ng/mL) for 4 hours to induce inflammatory signaling and co-treated with escalating dilutions of ST266 (1/128 to 1/4) in media. NF-κB activity was quantified by measuring luciferase luminescence and expressed as a percentage of the TNF-α–stimulated control (set to 100%). Data are presented as mean ± SEM. ST266 significantly suppressed NF-κB activation in a dose-dependent manner, with the strongest inhibition observed at 1/4 dilution. Statistical comparisons were made using one-way ANOVA with post hoc testing: *p < 0.05, **p < 0.01, ****p < 0.0001.

ST266 Attenuates NF-κB–Dependent Pro-Inflammatory Signaling in Activated Microglia



ST266 suppresses pro-inflammatory cytokine production in LPS-stimulated N9 microglia. N9 microglial cells were stimulated with LPS and co-treated with 10% or 20% ST266. ST266 significantly reduced TNF-α, IL-12p70, and IFN-γ secretion, supporting a concentration-dependent immunomodulatory effect. Data are mean ± SEM; one-way ANOVA with post hoc testing. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

ST266 Attenuates Chronic Neuroinflammation, and Gliosis following RHI



ANOVA table	F (DFn, DFd)	P value
Interaction	F (1, 32) = 3.857	P=0.0583
Injury	F (1, 32) = 55.14	P<0.0001
Treatment	F (1, 32) = 3.520	P=0.0698

Tukey's multiple comparisons test	Adjusted P Value
Sham:Vehicle vs. Sham:ST266	>0.9999
Sham:Vehicle vs. RHI:Vehicle	<0.0001
Sham:Vehicle vs. RHI:ST266	0.0023
Sham:ST266 vs. RHI:Vehicle	<0.0001
Sham:ST266 vs. RHI:ST266	0.0028
RHI:Vehicle vs. RHI:ST266	0.0493

ANOVA table	F (DFn, DFd)	P value
Interaction	F (1, 32) = 6.514	P=0.0157
Injury	F (1, 32) = 63.70	P<0.0001
Treatment	F (1, 32) = 10.72	P=0.0025

Tukey's multiple comparisons test	Adjusted P Value
Sham:Vehicle vs. Sham:ST266	0.9560
Sham:Vehicle vs. RHI:Vehicle	<0.0001
Sham:Vehicle vs. RHI:ST266	0.0113
Sham:ST266 vs. RHI:Vehicle	<0.0001
Sham:ST266 vs. RHI:ST266	0.0029
RHI:Vehicle vs. RHI:ST266	0.0014

ANOVA table	F (DFn, DFd)	P value
Interaction	F (1, 68) = 10.44	P=0.0019
Injury	F (1, 68) = 123.2	P<0.0001
Treatment	F (1, 68) = 13.28	P=0.0005

Tukey's multiple comparisons test	Adjusted P Value
Sham:Vehicle vs. Sham:ST266	0.9912
Sham:Vehicle vs. RHI:Vehicle	<0.0001
Sham:Vehicle vs. RHI:ST266	<0.0001
Sham:ST266 vs. RHI:Vehicle	<0.0001
Sham:ST266 vs. RHI:ST266	<0.0001
RHI:Vehicle vs. RHI:ST266	<0.0001

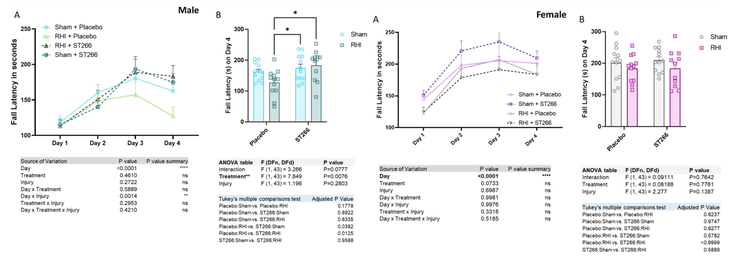
Fig. 5. CD68 burden in the corpus callosum 6 months after RHI. CD68-positive staining was quantified in a 200 µm² region of the corpus callosum in male, female, and combined-sex groups. RHI significantly increased CD68 expression versus sham controls, indicating persistent microglial/macrophage activation. ST266 significantly reduced CD68 burden after RHI, with consistent effects across sexes. Data are mean ± SEM; points represent individual animals. Significance is indicated on the graph.

In Vitro Assays

NF-κB reporter assay: HEK293 NF-κB-luciferase reporter cells were stimulated with TNF-α (25 ng/mL) and co-treated with ST266 dilutions ranging from 1/128 to 1/4 for 4 hours; NF-κB activity was measured by luciferase assay.

Microglial cytokine assay: N9 microglial cells were stimulated with LPS (100 ng/mL) for 24 hours and co-treated with **10% or 20% ST266**; cytokines were measured using MSD V-PLEX assay; **n = 8** for control and LPS-only groups and each ST266 treatment group.

The Rotarod test results demonstrate that ST266 treatment significantly enhances motor performance in male mice at 6 months post-injury, whereas no significant effect was observed in female mice.



Source of Variation	P value	P value summary
Day	<0.0001	****
Treatment	0.4610	ns
Injury	0.2722	ns
Day x Treatment	0.5889	ns
Day x Injury	0.0014	**
Treatment x Injury	0.2953	ns
Day x Treatment x Injury	0.4210	ns

ANOVA table	F (DFn, DFd)	P value
Interaction	F (1, 43) = 3.266	P=0.0777
Treatment**	F (1, 43) = 7.849	P=0.0076
Injury	F (1, 43) = 1.196	P=0.2803

Tukey's multiple comparisons test	Adjusted P Value
Placebo:Sham vs. Placebo:RHI	0.1778
Placebo:Sham vs. ST266:Sham	0.8822
Placebo:Sham vs. ST266:RHI	0.6335
Placebo:RHI vs. ST266:Sham	0.0282
Placebo:RHI vs. ST266:RHI	0.0125
ST266:Sham vs. ST266:RHI	0.9588

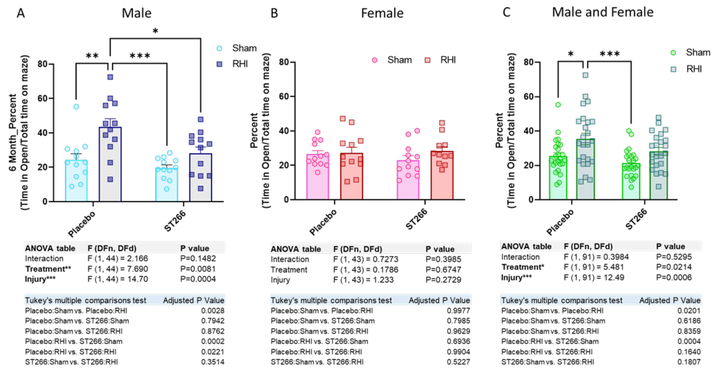
Source of Variation	P value	P value summary
Day	<0.0001	****
Treatment	0.0733	ns
Injury	0.6987	ns
Day x Treatment	0.6981	ns
Day x Injury	0.9976	ns
Treatment x Injury	0.5318	ns
Day x Treatment x Injury	0.5185	ns

ANOVA table	F (DFn, DFd)	P value
Interaction	F (1, 43) = 0.09111	P=0.7642
Treatment	F (1, 43) = 0.08188	P=0.7761
Injury	F (1, 43) = 2.277	P=0.1387

Tukey's multiple comparisons test	Adjusted P Value
Placebo:Sham vs. Placebo:RHI	0.8237
Placebo:Sham vs. ST266:Sham	0.9747
Placebo:Sham vs. ST266:RHI	0.8277
Placebo:RHI vs. ST266:Sham	0.8782
Placebo:RHI vs. ST266:RHI	>0.9999
ST266:Sham vs. ST266:RHI	0.6889

Fig 6: Rotarod Performance of Female mice tested over four days at 6 Months Post-Injury. **(A)** All groups show improvement from Day 1 to Day 3, with fall latency peaking on Day 3. On Day 4, fall latency levels off with no substantial differences observed between the groups. (**B**) No significant differences in performance are observed between any of the groups on Day 4. (A) Three Way ANOVA, (B) Data are presented as mean ± SEM, Two-way analysis of variance with Tukey's post hoc test; P > 0.05; each symbol represents 1 mouse.

The findings suggest that ST266 treatment reduces disinhibition-like behavior (an increase time spent in an open arm means less anxiety) in male mice with RHI, indicating a strong therapeutic effect. In contrast, no significant effects of RHI or ST266 treatment were observed in female mice, suggesting a possible sex-specific response at 6 months post RHI.



ANOVA table	F (DFn, DFd)	P value
Interaction	F (1, 44) = 2.166	P=0.1482
Treatment**	F (1, 44) = 7.690	P=0.0081
Injury***	F (1, 44) = 14.70	P=0.0004

Tukey's multiple comparisons test	Adjusted P Value
Placebo:Sham vs. Placebo:RHI	0.0028
Placebo:Sham vs. ST266:Sham	0.7942
Placebo:Sham vs. ST266:RHI	0.8762
Placebo:RHI vs. ST266:Sham	0.0002
Placebo:RHI vs. ST266:RHI	0.0221
ST266:Sham vs. ST266:RHI	0.3514

ANOVA table	F (DFn, DFd)	P value
Interaction	F (1, 43) = 0.7273	P=0.3985
Treatment	F (1, 43) = 0.1786	P=0.6747
Injury	F (1, 43) = 1.233	P=0.2729

Tukey's multiple comparisons test	Adjusted P Value
Placebo:Sham vs. Placebo:RHI	0.9977
Placebo:Sham vs. ST266:Sham	0.7985
Placebo:Sham vs. ST266:RHI	0.9629
Placebo:RHI vs. ST266:Sham	0.6936
Placebo:RHI vs. ST266:RHI	0.9904
ST266:Sham vs. ST266:RHI	0.5227

ANOVA table	F (DFn, DFd)	P value
Interaction	F (1, 91) = 0.3984	P=0.5295
Treatment*	F (1, 91) = 5.481	P=0.0214
Injury***	F (1, 91) = 12.49	P=0.0006

Tukey's multiple comparisons test	Adjusted P Value
Placebo:Sham vs. Placebo:RHI	0.0201
Placebo:Sham vs. ST266:Sham	0.6186
Placebo:Sham vs. ST266:RHI	0.8359
Placebo:RHI vs. ST266:Sham	0.0004
Placebo:RHI vs. ST266:RHI	0.1640
ST266:Sham vs. ST266:RHI	0.1807

Fig 7. Assessment of risk like behavior at 6 months Post-RHI prior euthanasia. The figure shows the percent time spent in the open arms of the Elevated Plus Maze for the males (A), Female (B) and combined male and females (C). Each graph includes an ANOVA table and the Tukey's multiple comparisons test results. There is a significant injury and treatment effect observed in male (A) but not in female mice (B). With both sex combined results this difference is still here but less significant (C). VA-Tukey; data are presented as Whiskers plots: Min to Max. Each symbol represents 1 mouse *N* = 10/12 per group).

Conclusions

Neuropathology
- Six months post-injury: **persistent axonal injury, astrogliosis, & microglial infiltration**
- CD45 & CD68 burden reduced across brain regions **consistent anti-inflammatory effects**

Mechanistic Basis
- **Dose-dependent suppression of NF-κB signaling** in vitro
- Multi-targeted cytokine modulation in activated microglia:
  - ↓ TNF-α, IL-12p70, IFN-γ (pro-inflammatory)

Functional Improvements
- Enhanced motor performance on Rotarod (male mice at 6 months post-injury)
- Reduced anxiety-like behavior (Elevated Plus Maze)

Sex-consistent neuropathological benefits across both males & female

Clinical Significance & Future Directions

I. ST266 effective as a **delayed therapeutic intervention** administered 1M post injury

II. Addresses critical unmet need: most concussion treatment occurs **after symptom emergence**

III. Potential translational application for **chronic post-concussive pathology in Veterans & civilian**

IV. Supports continued investigation of ST266 as a neuroprotective therapeutic for RHI

Acknowledgments

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