neuren pharmaceuticals

Development of NNZ-2566 for Mild TBI

Arrowhead TBI Conference

March 2012

Acknowledgement and Forward Looking Statement

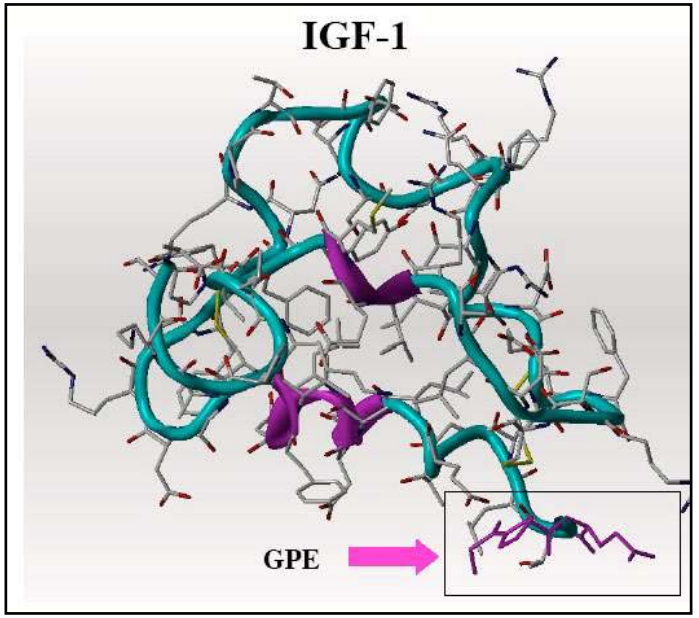
Research described in this presentation has been partially funded by the U.S. Army Medical Research and Materiel Command (grant number W81XWH-09-1-0496). The opinions expressed are not necessarily endorsed by the U.S. Army.

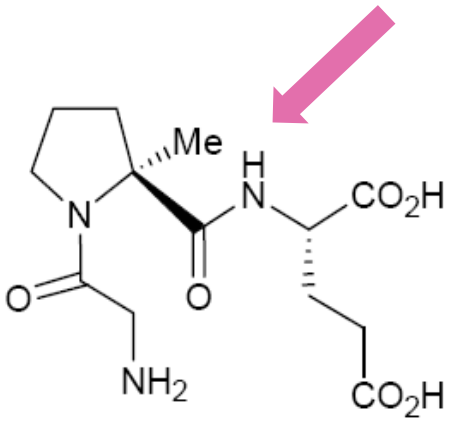
This presentation contains forward looking statements that involve risks and uncertainties. Although we believe that the expectations reflected in the forward looking statements are reasonable at this time, Neuren can give no assurance that these expectations will prove to be correct.

Actual results could differ materially from those anticipated. Reasons may include risks associated with drug development and manufacture, risks inherent in the regulatory processes, delays in clinical trials, risks associated with patent protection, future capital needs or other general risks or factors.

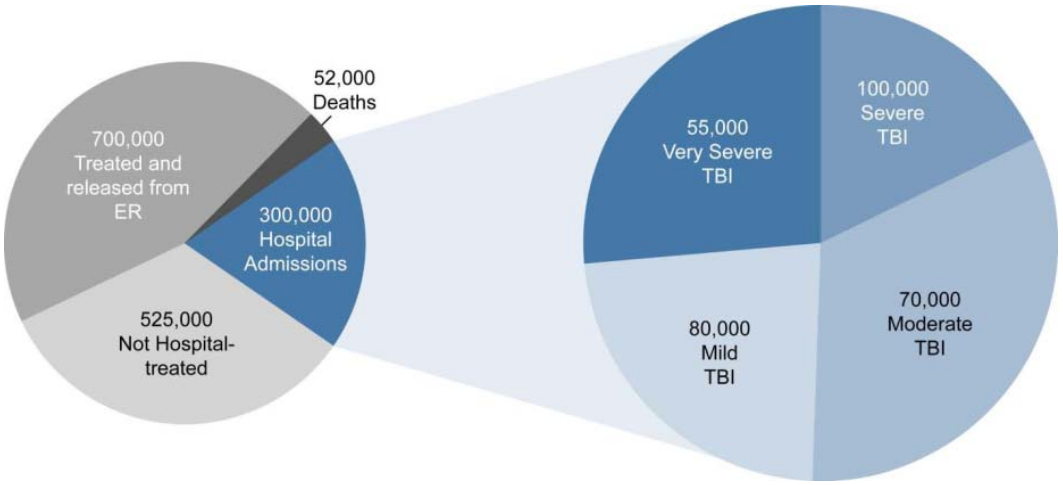
NNZ-2566 in mild TBI: an “addressable” indication

- Unmet medical need
- Biological rationale
- Oral bioavailability and CNS penetration
- Acceptable safety profile
- Feasible clinical trial
- Clear regulatory pathway





Unmet need

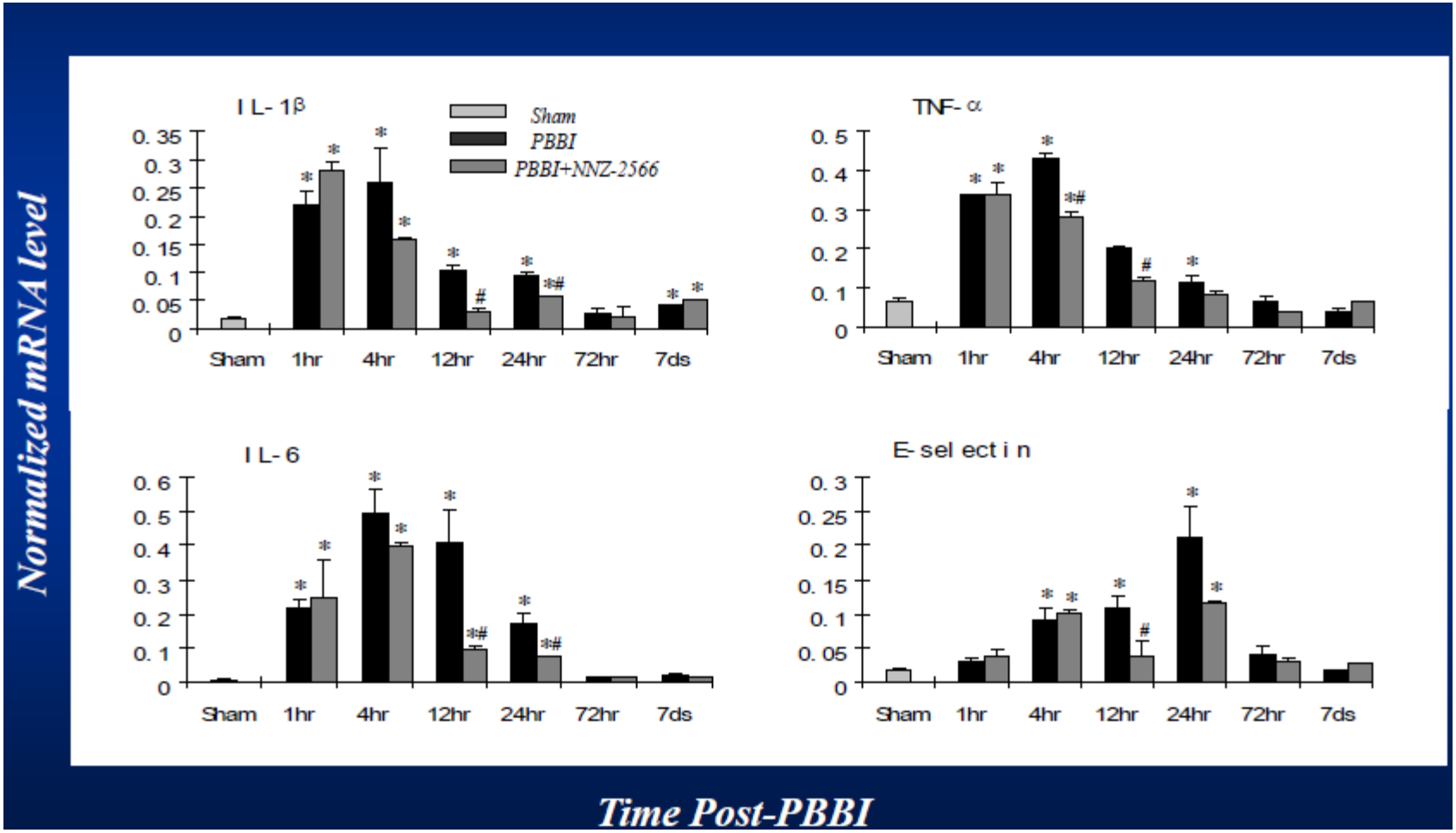


- >1 million cases of mTBI in the US annually
- >168,000 in the military from 2001 – 2011 (77.6% of total reported TBI)
- Post-concussive symptoms affect up to 50% of mTBI patients at 1 month and 15-25% at 1 year
- Symptoms can include impaired cognitive performance, memory and attention, mood disturbance, sleep disorders and fatigue and can cause significant functional disability and reduced quality of life
- No approved pharmacotherapy

Biological rationale

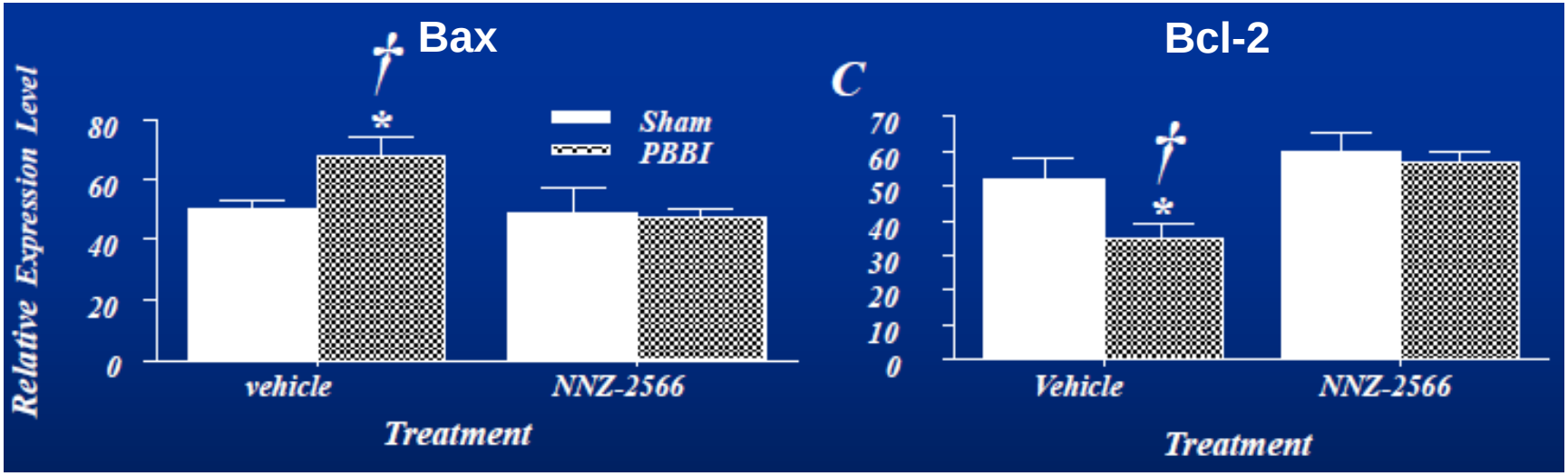
- mTBI can result in a wide range of molecular and cellular effects
  - Up-regulation of inflammatory cytokine expression
  - Up-regulation of pro-apoptotic gene expression
  - Down-regulation of anti-apoptotic gene expression
  - Astrogliosis
  - Microglial activation
  - EEG abnormalities
  - Leukocyte infiltration
  - Deficits in synaptic plasticity
- NNZ-2566 (IV and oral) has been tested in a range of *in vivo* models
  - Endothelin-induced MCAO
  - Transient MCA ligation
  - Global hypoxia-ischemia
  - Neonatal hypoxia-ischemia
  - pMCAO non-convulsive seizure
  - Penetrating TBI
  - Cortical concussive TBI
  - Aged rat
  - MeCP2 (Rett Syndrome) mouse

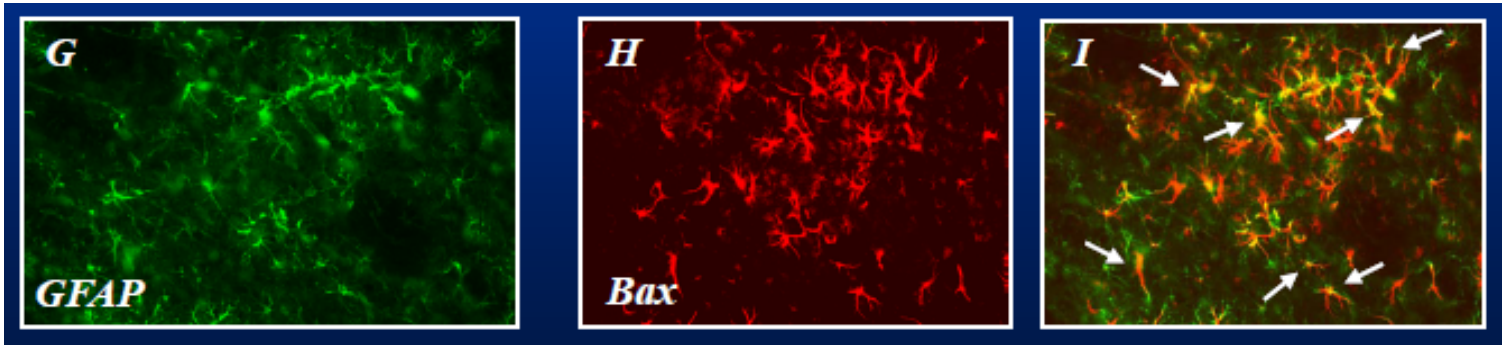
NNZ-2566 attenuates expression of inflammatory cytokines and adhesion molecules



Wei, et. al., J Neuroinflammation (2009)

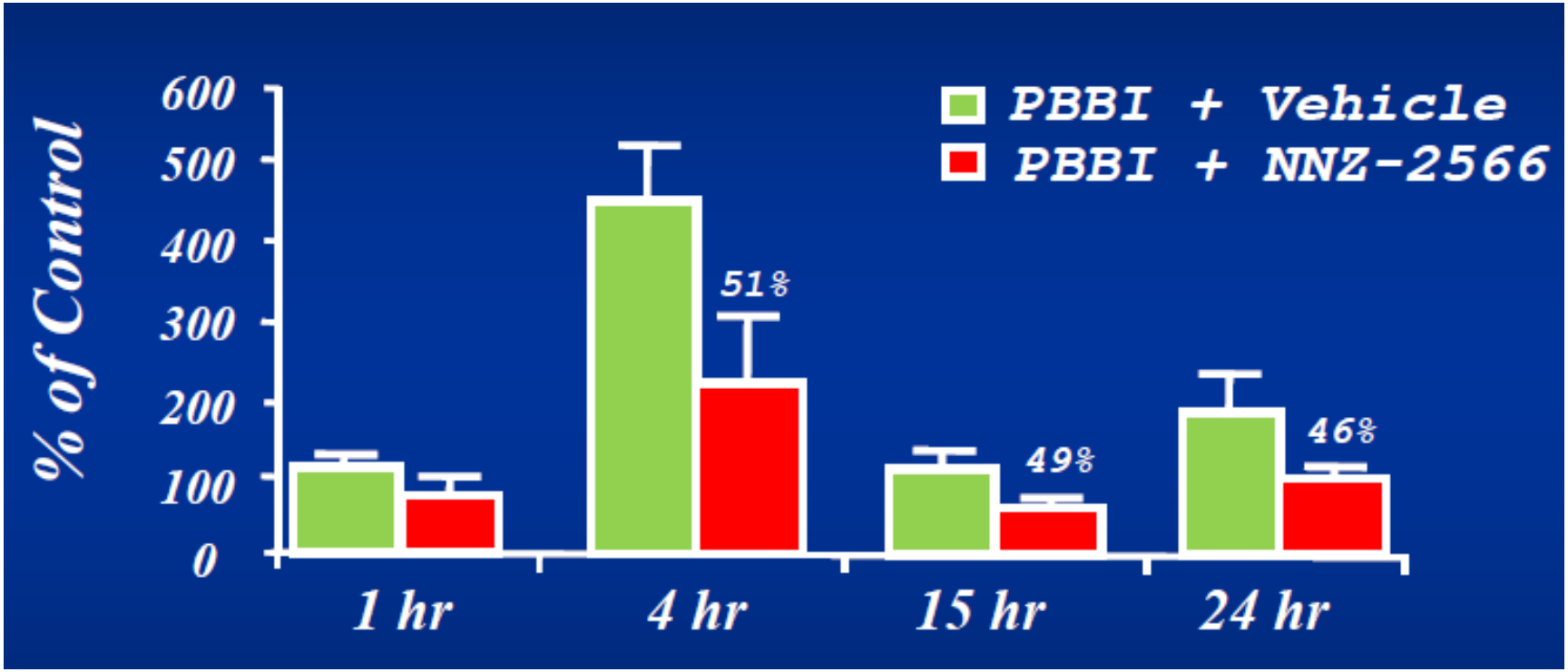
NNZ-2566 normalizes Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic) expression; Bax co-localized with astrocytes





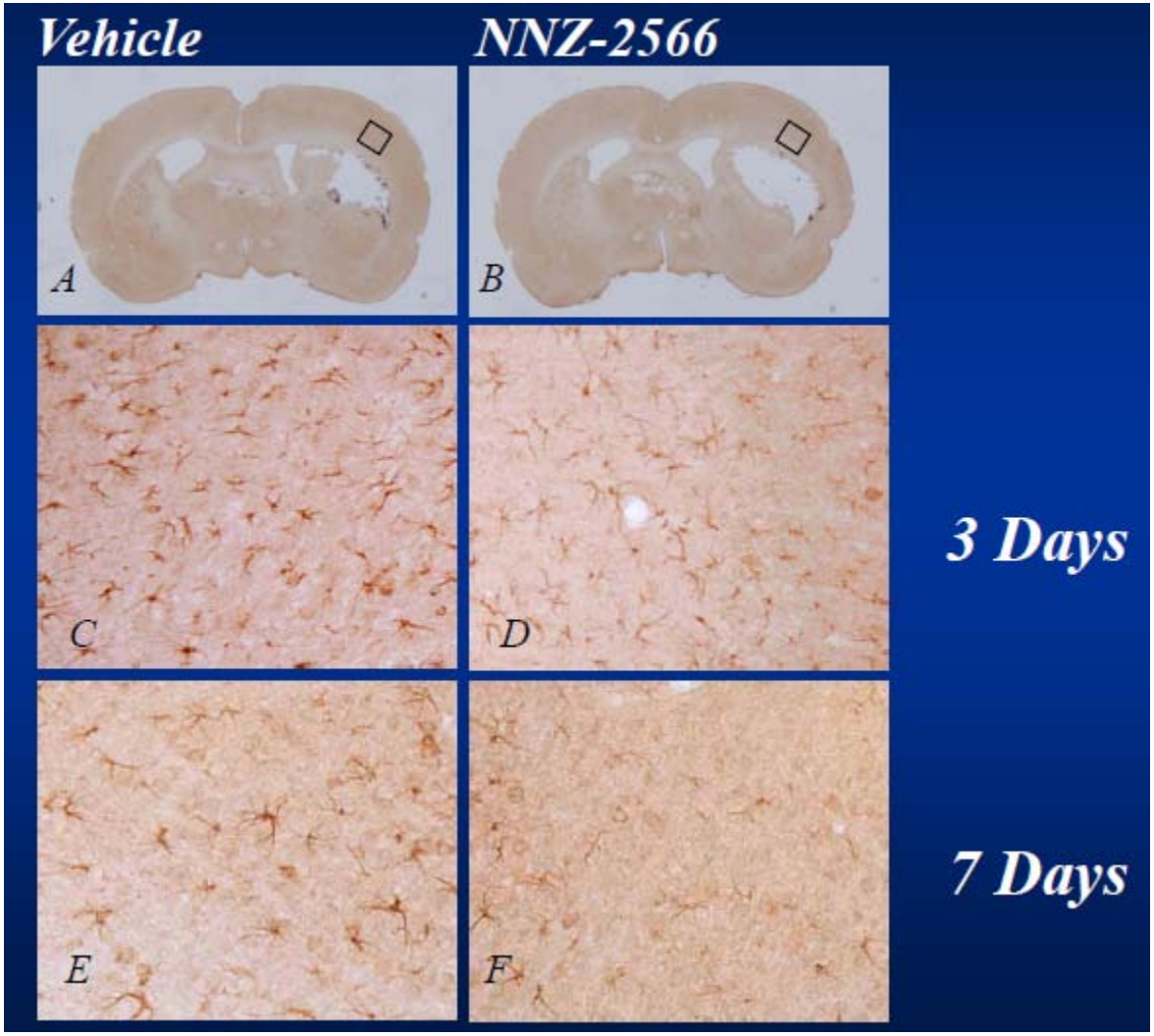
Lu, et. al., J Neurotrauma (2009)

Caspase-3 activity is reduced by NNZ-2566



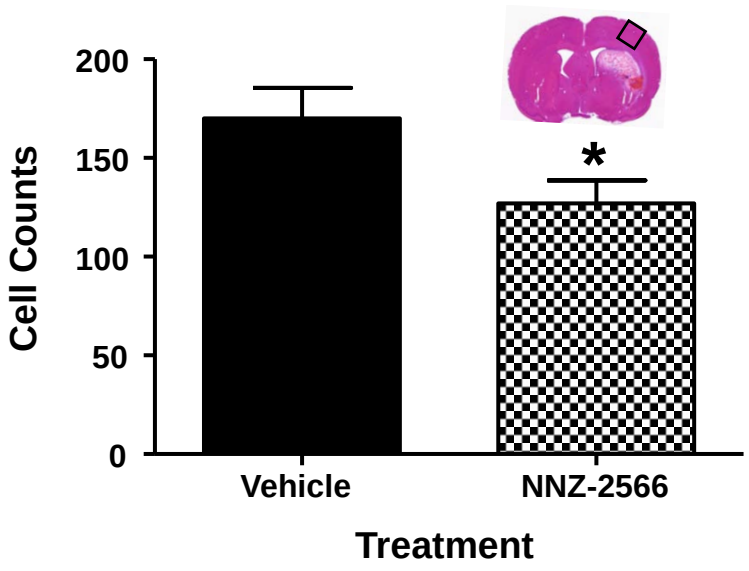
Lu, et. al., J Neurotrauma (2009)

NNZ-2566 inhibits reactive gliosis: IL-1β immunoreactivity localized to microglia

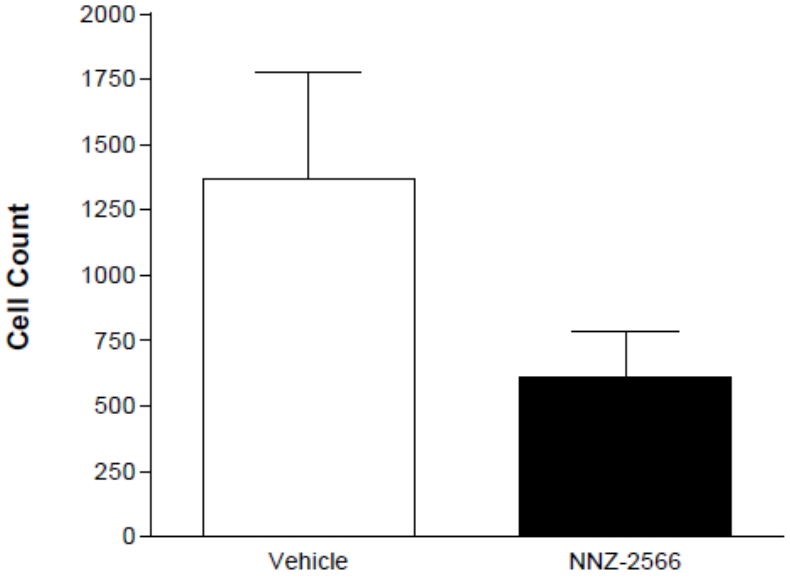


Wei, et. al., J Neuroinflammation (2009)

NNZ-2566 inhibits microglial activation



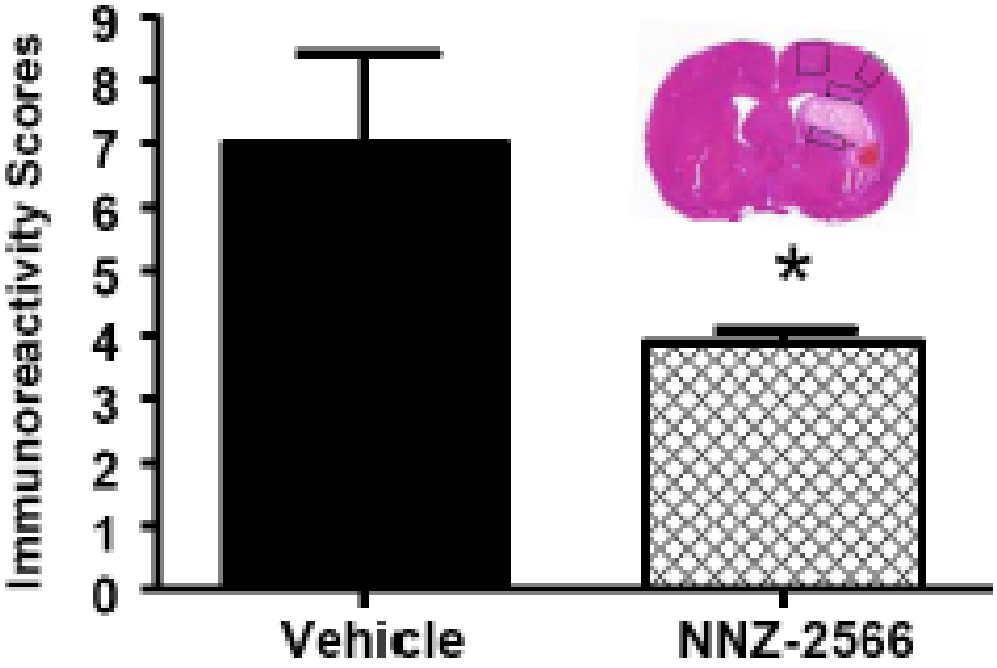
PBBI Model



MCAO Model

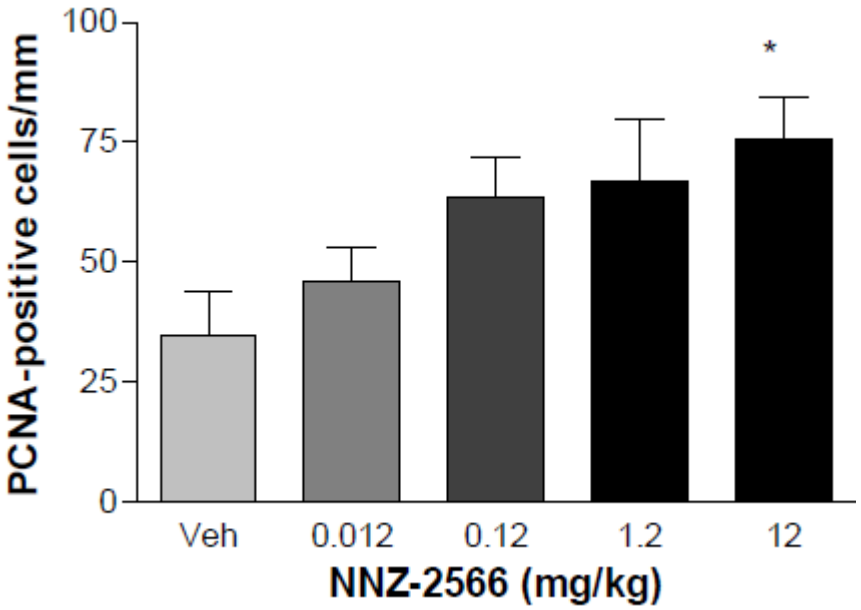
Lu, et. al., J Neurotrauma (2009)

NNZ-2566 inhibits leukocyte infiltration

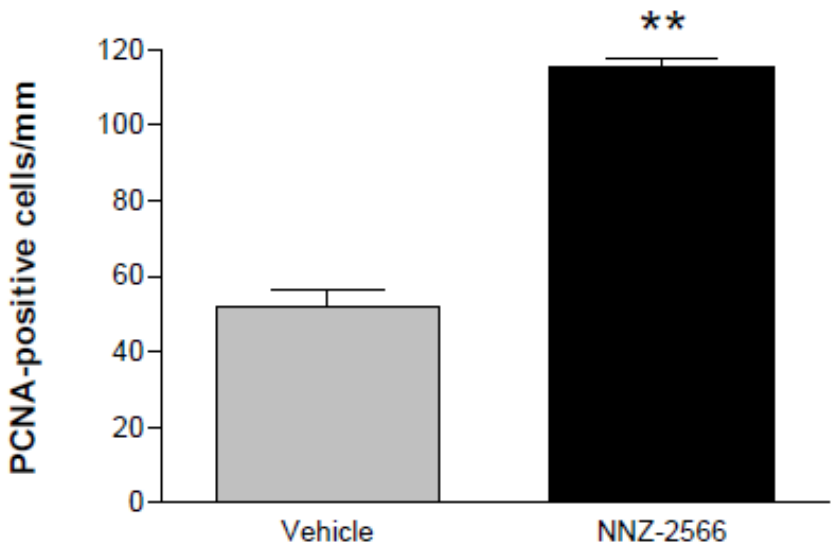


Lu, et. al., J Neurotrauma (2009)

Increased cellular proliferation in the subventricular zone of aged rat brains

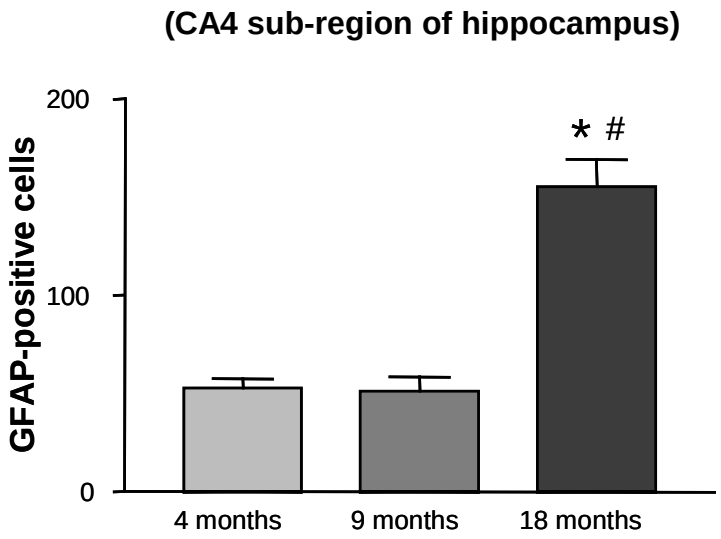


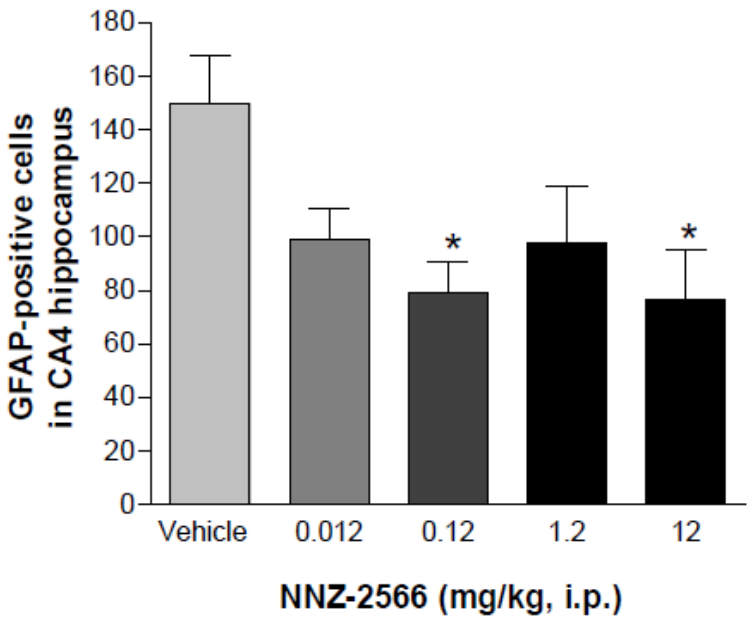
18-month old rats

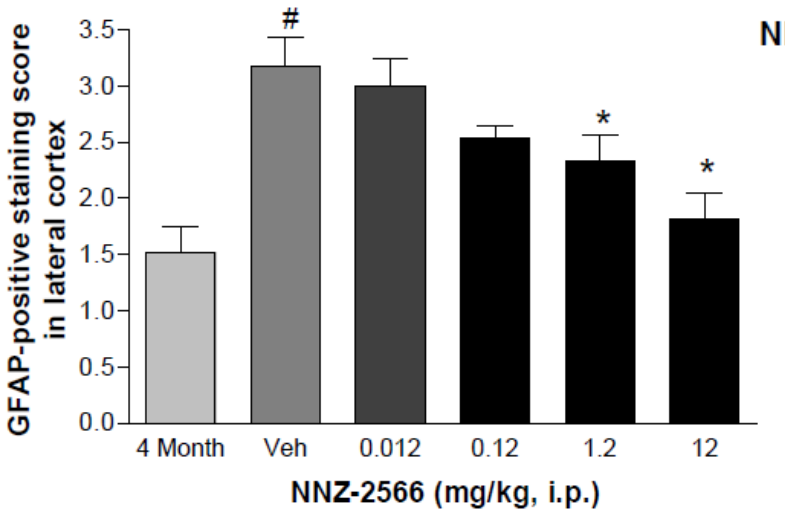


9-month old rats

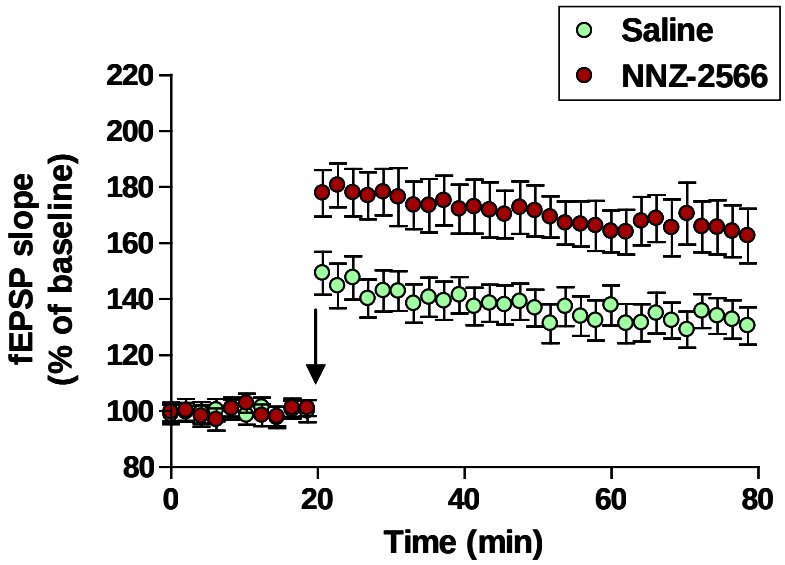
Reduction of reactive astrocytes in the brains of aged rats

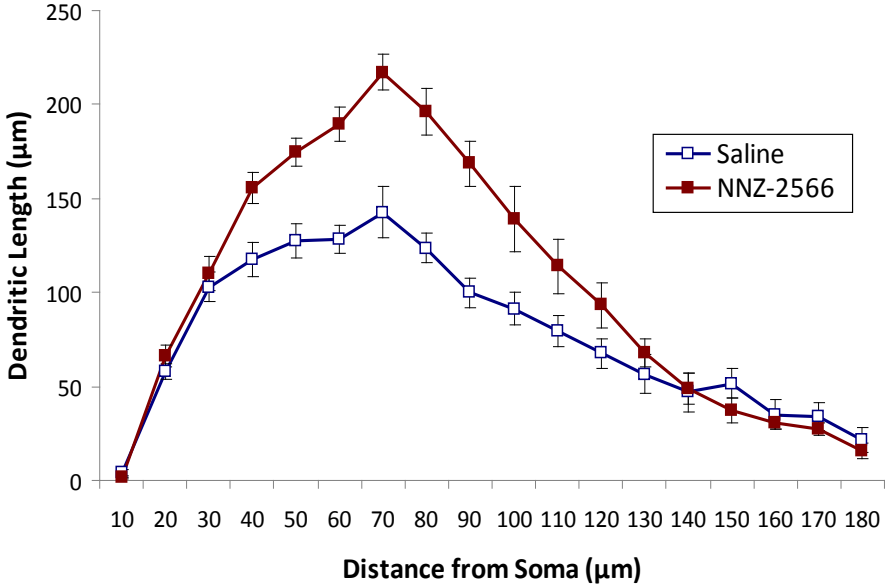






Effects on synaptic plasticity in the MeCP2 model

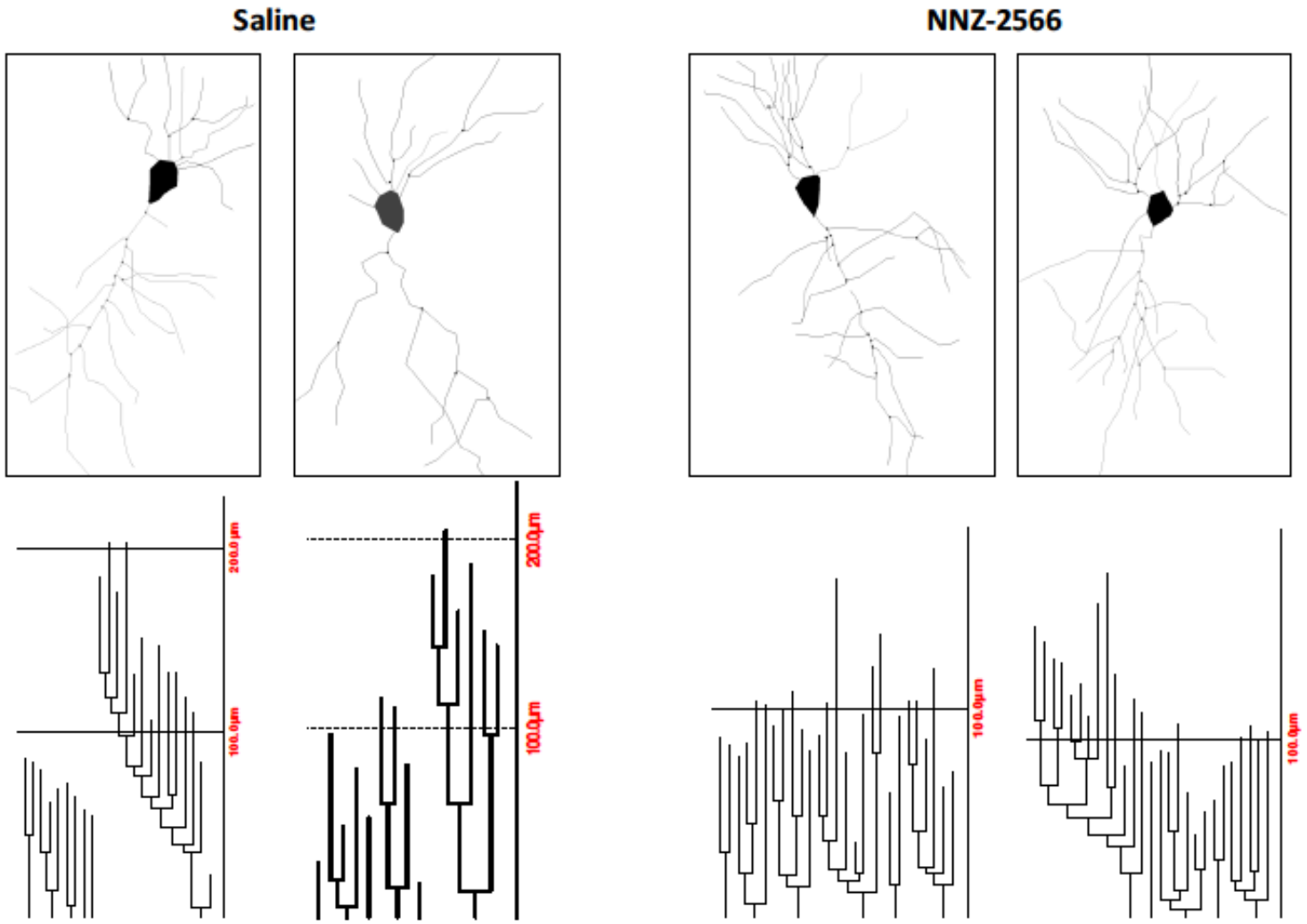




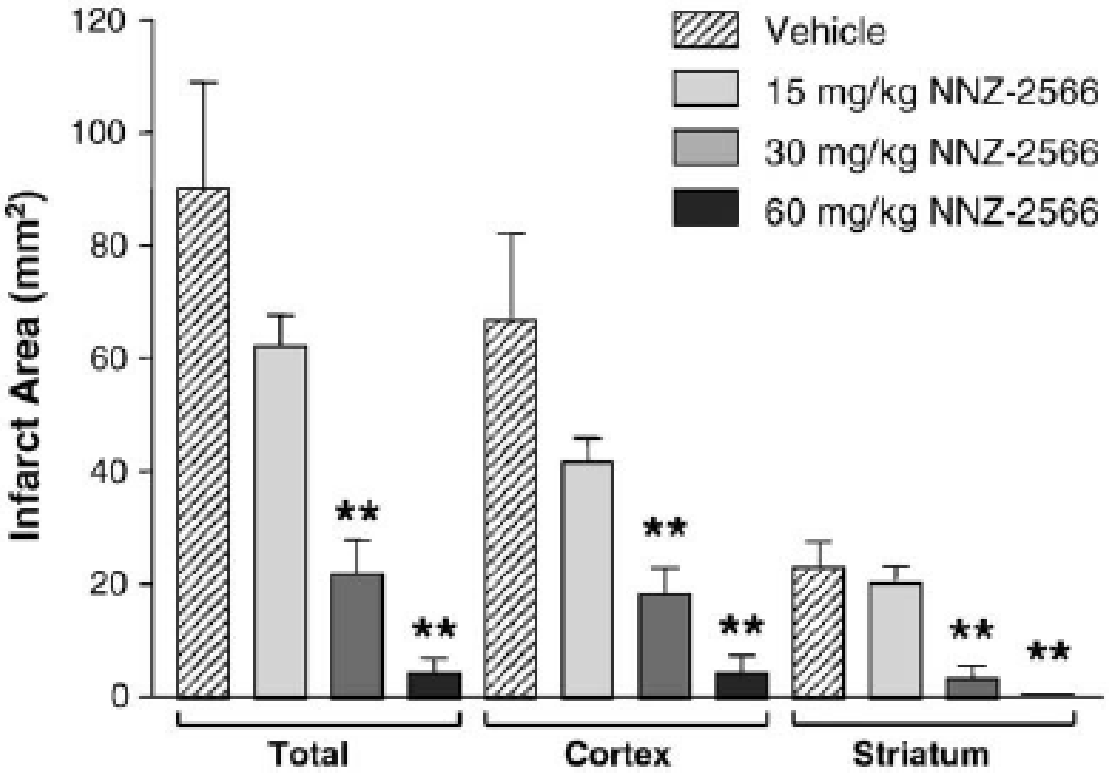
NNZ-2566 (20 mg/kg i.p. 1/day) increases hippocampal LTP in the *mecp2* mouse model of Rett syndrome (Jaenisch *mecp2*$^{y/-}$ knockout).

NNZ-2566 increases mean dendrite length in the CA1 region of the hippocampus in *mecp2* mutant mice.

Increased dendritic branching in the MeCP2 model

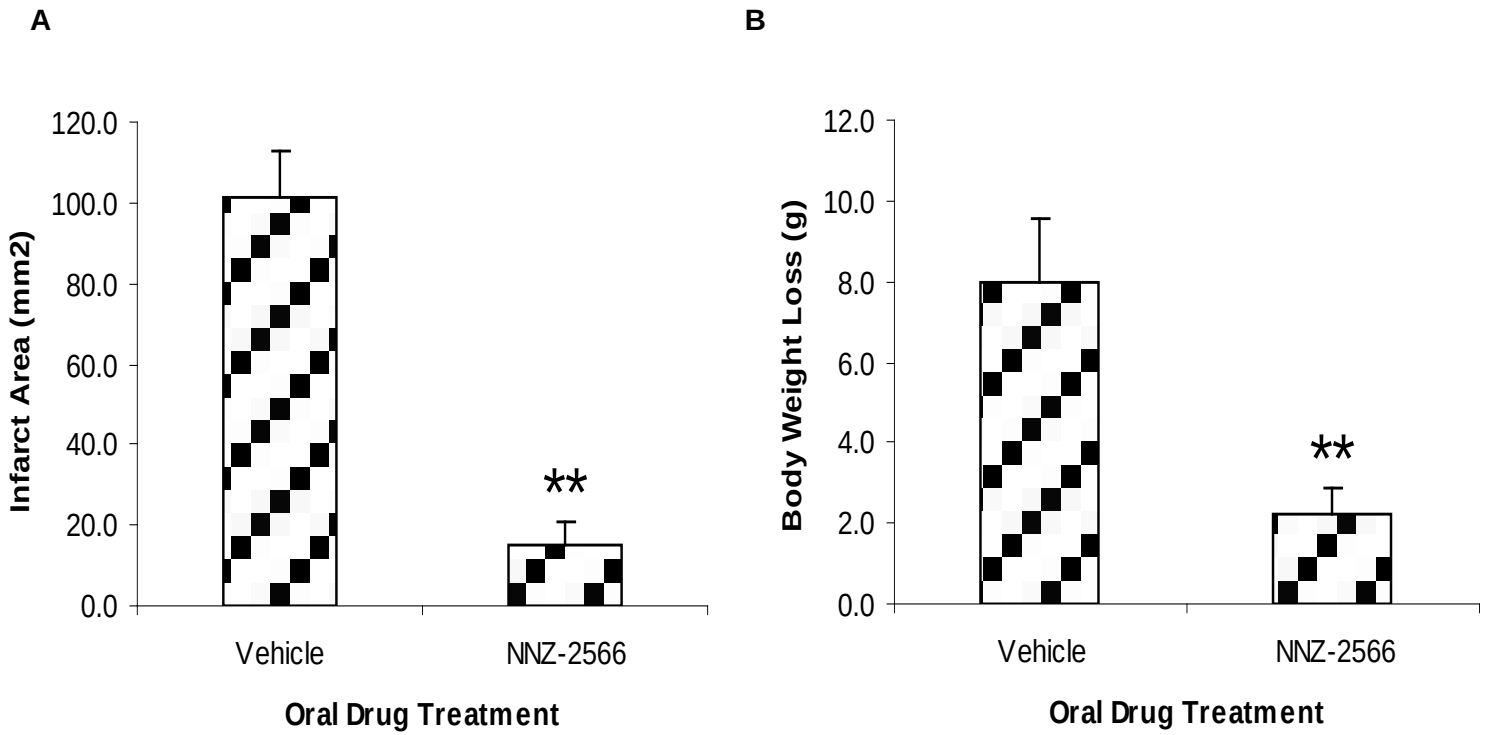


Dose-dependent reduction in brain injury with single oral dose at 3 hrs



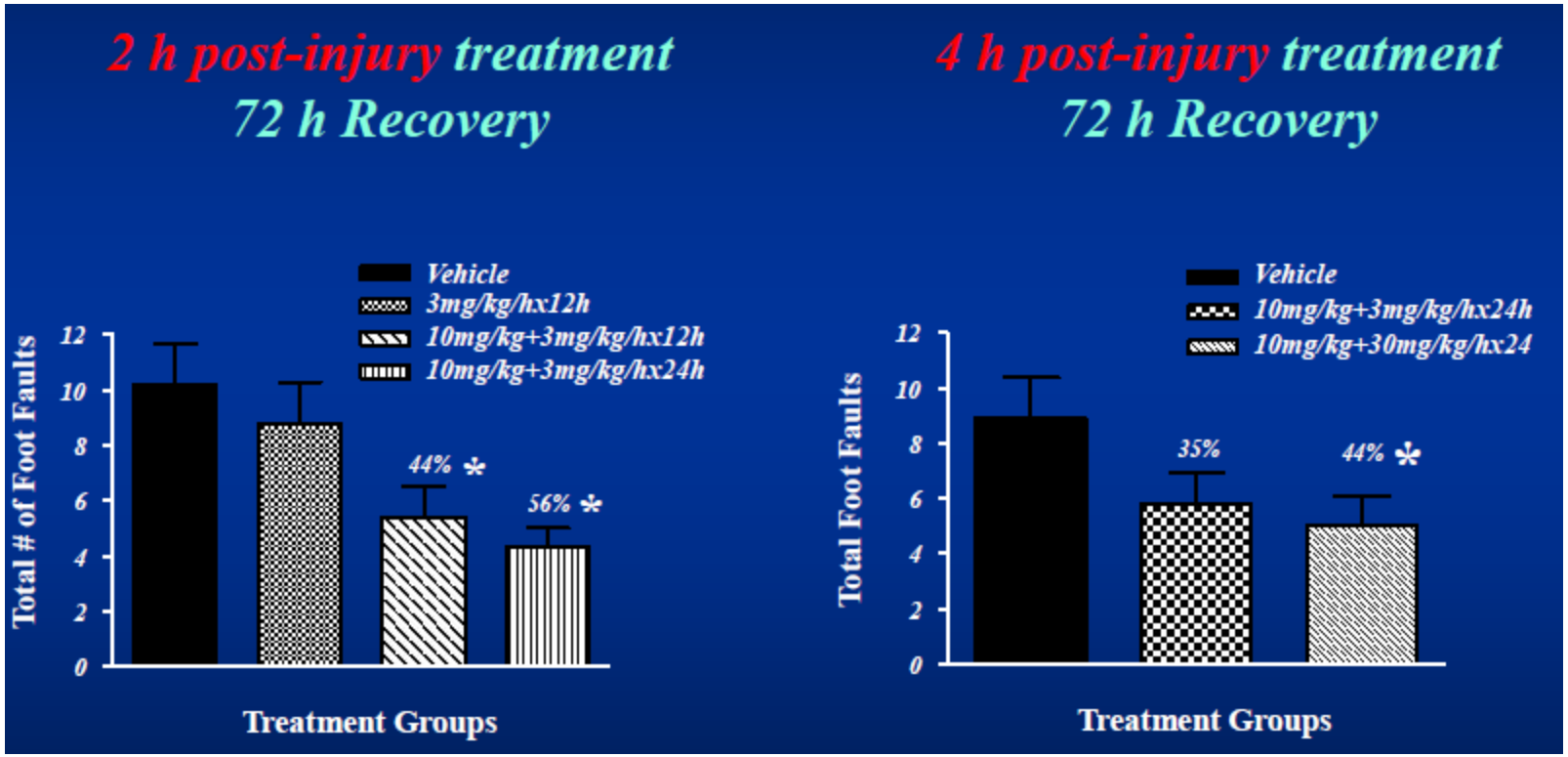
Bickerdike, et. al., J Neurological Sciences (2009)

Reduction of brain injury and weight loss with two oral doses at 2 and 4 hrs following injury (MCAO model)



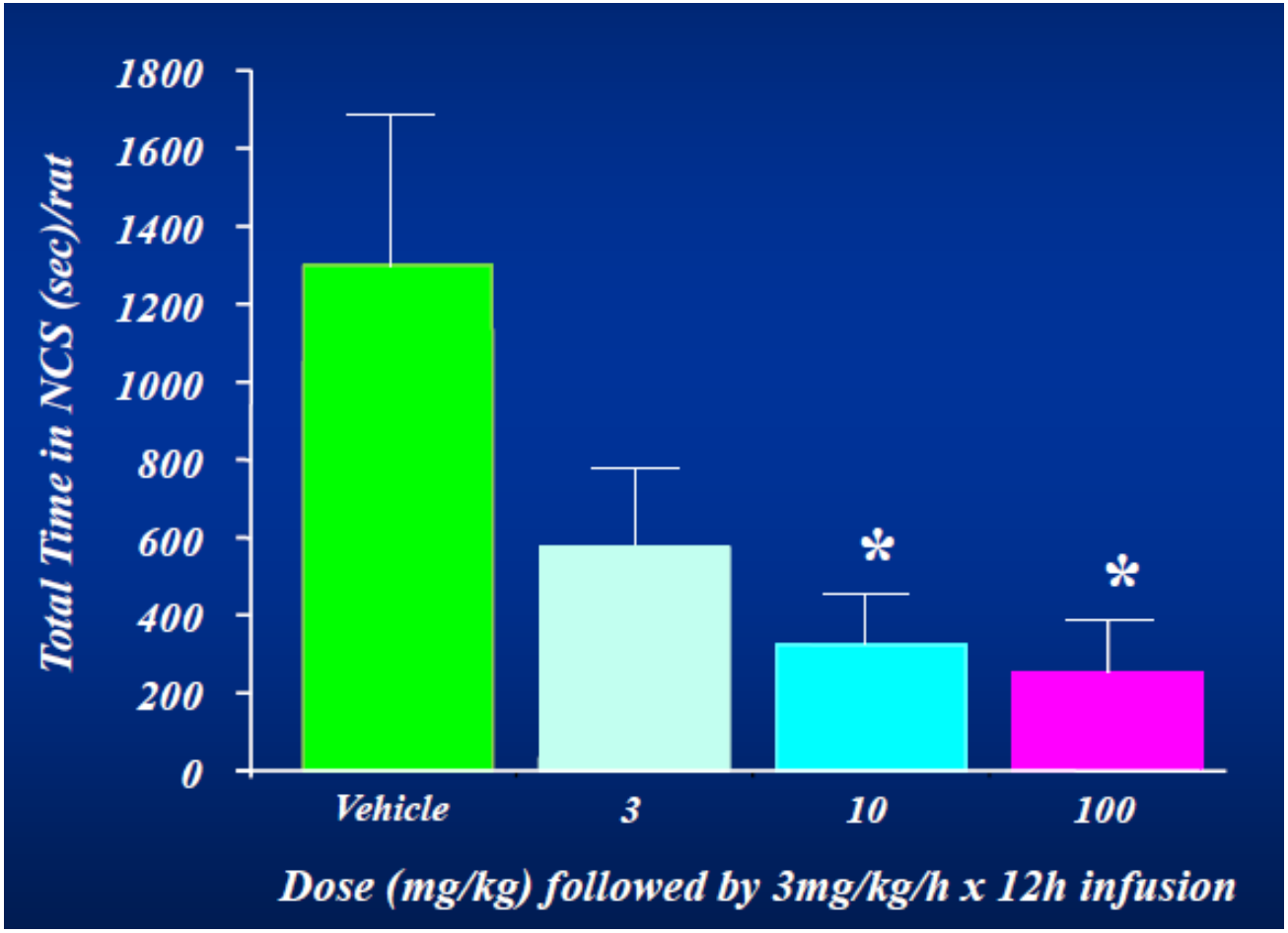
40 mg/kg by oral gavage

Dose-dependent improvement in neurofunction decrement (foot faults)



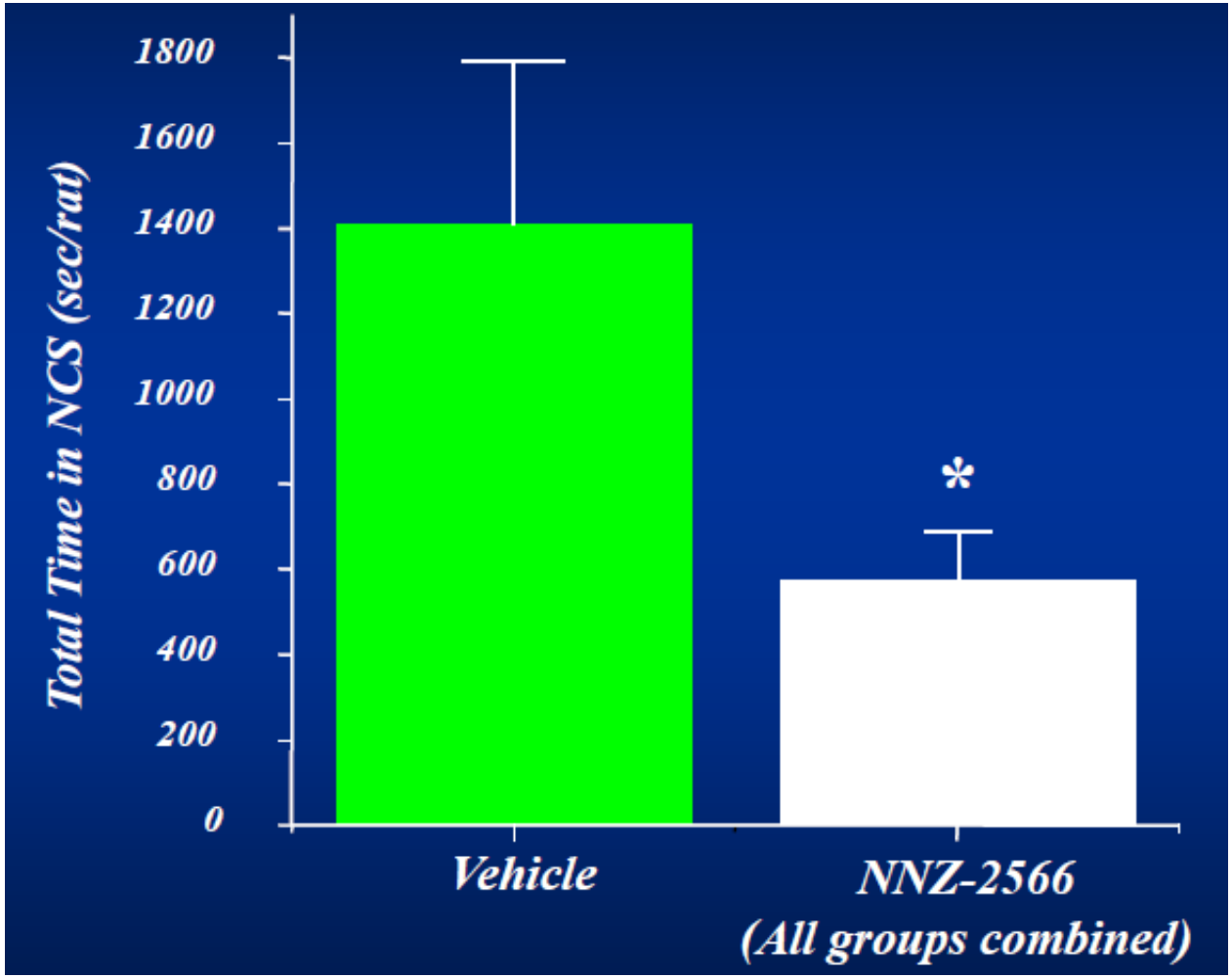
Lu, et. al., J Neurotrauma (2009)

Dose-dependent attenuation of post-injury non-convulsive seizure activity



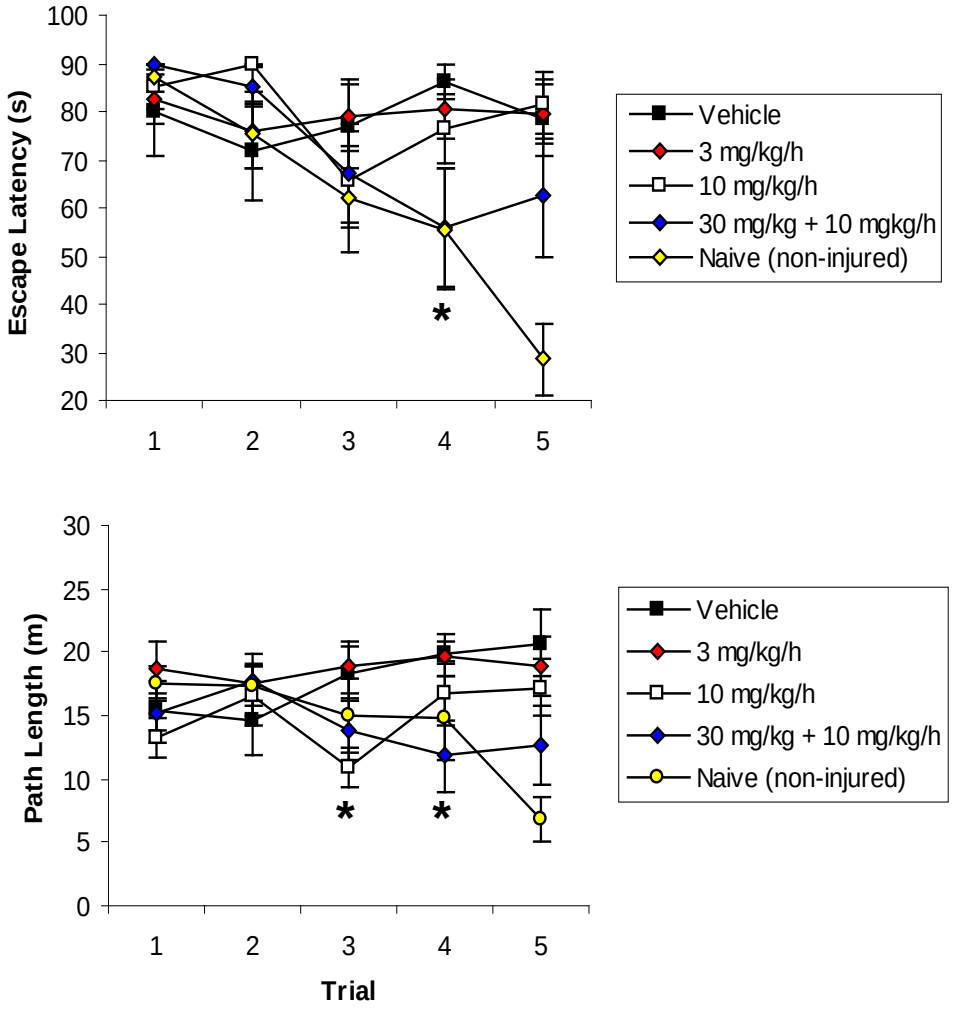
Lu, et. al., JCBFM (2009)

Attenuation of post-injury non-convulsive seizures after onset

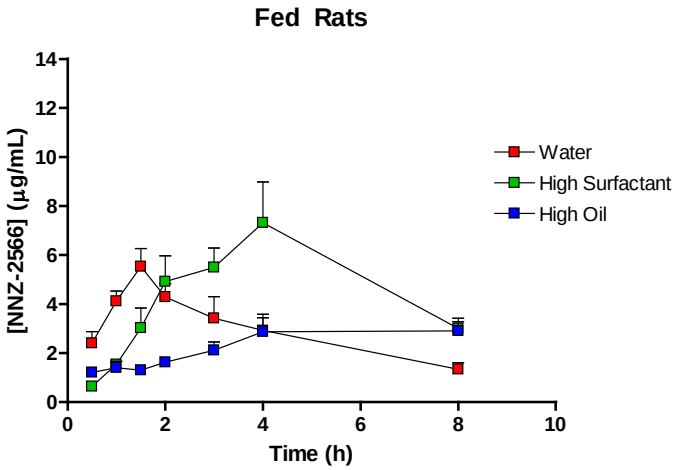


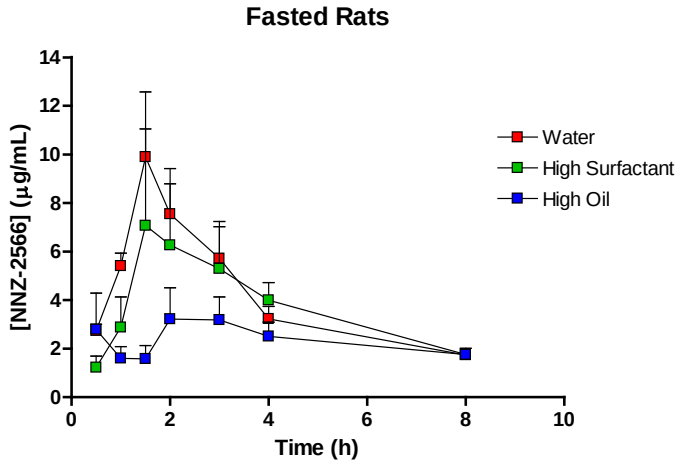
Lu, et. al., JCBFM (2009)

Improved working memory (Morris water maze) in a cortical impact model at 28 days

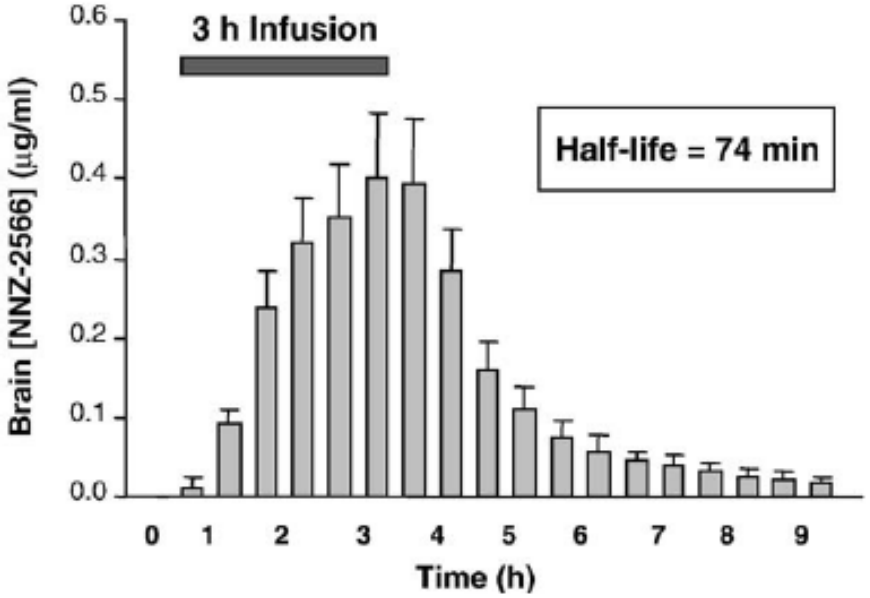


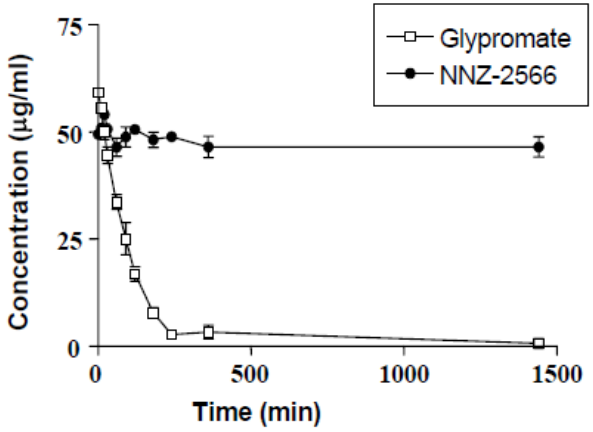
Oral bioavailability and CNS penetration





T_{max} at <2 hours with aqueous formulation





Enzymatic stability in Caco-2 monolayer

Safety profile: non-clinical

- Safety pharmacology – no or insignificant effects on:
  - Genotoxicity (Ames, chromosome aberration, micronucleus)
  - hERG channel assays
  - Respiratory safety pharmacology
  - Irwin screen (rat neurobehavioral)
  - Reproductive toxicology (Segment I) – Mating fertility and fecundity in male or female rats
  - Drug interaction studies (CYP-450 inhibition, CYP-450 induction, p-glycoprotein transport interaction)
- Metabolism
  - Resistant to proteolytic degradation in Caco-2 cells; stable in plasma; limited degradation in liver microsomes
- Single-dose toxicity studies (IV)
  - 500 mg/kg tolerated in mice
  - 350 mg/kg tolerated in rats
  - 175 mg/kg tolerated in dogs (12 x C_{max} in humans following 20 mg/kg bolus)
- Repeat dose toxicity studies (IV)
  - 700 mg/kg/day x 14 days tolerated in rats
  - 1440 mg/kg/day x 28 days tolerated in dogs (7 x total daily exposure in humans at 20 mg/kg bolus followed by 24 hours at 6 mg/kg/hour)
- 28-day bridging toxicology study (oral)
  - No adverse effects at 50, 400 or 700 mg/kg TID (NOAEL = 2100/mg/kg/day)

Safety profile: clinical

- Four Phase I studies in healthy volunteers (intravenous formulation)
  - 3 studies in males, 1 study in females
  - 106 total volunteers (77 exposed to NNZ-2566)
  - Doses up to 20 mg/kg bolus followed by 6 mg/kg/hr x 72 hours
  - No SAEs
  - All AEs were mild or moderate; moderate AEs were predominantly infusion site reactions
- Phase I study in healthy male and female volunteers (oral formulation)
  - Four cohorts (2 single dose, 2 repeat dose)
  - Top dose: 30 mg/kg TID x 5 days
  - Study underway
- Phase II clinical trial in patients with moderate to severe TBI
  - Dose escalation design with patients randomized 2:1 (test article : placebo)
  - Three cohorts: all receive 20 mg/kg bolus followed by 1, 3 or 6 mg/kg/hr x 72 hours
  - Two SAEs reported as possibly or probably related to drug (same patient 3 days post end of infusion)
  - No effects observed on cardiac function or liver enzymes
  - DSMC review at completion of cohorts 1 and 2 found no safety concerns
  - Cohort 3 (highest dose) underway; no possibly or probably drug-related SAEs reported to date

Feasibility of clinical trials: challenges

- High placebo response rate (>50% of mTBI patients recover within 1 month)
- Heterogeneous population (type and extent of injury, age, sex, previous history of mTBI, baseline neurocognitive performance, other risk factors)
- Relying on normative data to determine post-injury neurocognitive deficit reduces sensitivity
- Frequent delay in presentation to emergency room
  - Difficulty completing screening and informed consent within defined therapeutic window (8 hours)
  - Reduces likelihood of drug administration within defined therapeutic window
  - May result in biased patient sample
- Consent by Legally Authorized Representative (LAR) likely will be required for some participants

Clinical trial feasibility: Phase II trial overview

- Randomized, double-blind, placebo and true (musculoskeletal injury) control
- Investigational product: 2 g or 3 g (based on weight) liquid dose TID for 5 days
- Single site: University of Pittsburgh Sports Medicine Concussion Program
  - PI: A. Kontos; Investigators: M. Collins, A. Mucha, N. Kegel, R. Elbin; Neurosurgeon: D. Okonkwo
- Assessment of neurocognitive performance, vestibular function and symptoms at time of injury, 5-7 days, 10-14 days and 1 month
- Patients enrolled from among those with baseline (pre-injury) neurocognitive assessment completed and participating in concussion assessment program
- Preliminary screening conducted pre-hospital by trained staff at site of injury
- Assessments
  - **Safety**
  - **MRI/DTI within 24 hours of injury**
  - **Serum biomarkers (Banyan Biomarkers and S100β)**
  - **Immediate Post-concussion Assessment and Cognitive Test (ImPACT) – neurocognitive performance and post-concussion symptoms**
  - **Vestibular function**
- Efficacy endpoint = time to recovery (return to baseline neurocognitive function and symptom-free at rest; symptom-free following exertion)

Regulatory pathway: assumptions

- Strong evidence of efficacy with acceptable safety in adequately powered Phase II will support progression to pivotal trial
- Time to recovery is an approvable endpoint
- ImPACT is acceptable as a primary outcome measure
- No *a priori* standard for magnitude of effect
- Two pivotal trials will be required for registration
- Dose-ranging assessment can be undertaken in first pivotal trial
- Pediatric patients can be included in pivotal trials

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