NNZ-2566 in Rett Syndrome and Autism Spectrum Disorders – Role and Update
Overview

• The natural growth factor IGF-1 is broken down in the body to IGF-1[1-3]

• NNZ-2566 is an analogue of IGF-1[1-3] developed by Neuren Pharmaceuticals Ltd.

• NNZ-2566 has enhanced oral availability and a pharmaceutical profile suitable for investigation in autism spectrum disorders.

• Autism, Rett Syndrome and Fragile X Syndrome: disorders of synaptic connectivity involving neuroinflammation

• IGF-1[1-3] and NNZ-2566 have benefit in models of Rett Syndrome and Fragile X Syndrome

• Clinical study in Rett Syndrome is underway

• Clinical study in Fragile X Syndrome is being planned
IGF-1, IGF-1[1-3] and NNZ-2566
IGF-1 and IGF-1[1-3]

- IGF-1 is essential for brain development, widely expressed in the CNS, upregulated following brain injury
- Naturally occurring neurotrophic factor – one of the brain’s self-repair mechanisms
- IGF-1[1-3] is believed to be the active neuroprotective moiety in response to injury
- IGF-1 [1-3] appears to upregulate IGF-1 in the brain, enhance IGF1R phosphorylation and expression of synaptic markers
IGF-1[1-3] Mechanism of Action

- IGF-1[1-3]:
  - Reduces cytokines\(^2\) and neuroinflammatory markers in brain
  - Activates Akt-mToR pathway in microglia
  - Increases markers of presynaptic and postsynaptic synapses
  - Activates Akt-mToR pathway in *mecp2* knockout mouse model of Rett Syndrome
  - IGF[1-3] reduces number of microglia in hippocampus following hypoxia ischemia in rat brain

---


Tropea et al. (2009) PNAS 106:2029
NNZ-2566 (oral formulation)

- NNZ-2566 is a (1-3)IGF-1 analog
  - Longer half-life
  - Crosses blood brain barrier
  - Orally available (45-50%)
  - Conventional solution phase synthesis
  - Validated manufacturing process
  - Excellent stability
- Targets: inflammation, synaptic function, and microglial activation
- Dose-dependent effects in animal models with delayed administration
- No clinical toxicity in rats at highest dose tested for 28 days
- No SAEs, no clinically significant AEs in Phase I trial up to 100 mg/kg b.i.d. x 5 days
- Formulated with water
  - Peak plasma level at 2 hours with or without food
- Creates opportunities for chronic dosing
Autism, Rett Syndrome and Fragile X Syndrome
Synapsopathy in ASDs

- Altered synapses in idiopathic (non-syndromal) and syndromal autism
- Functional consequences:
  - impairments in synaptic plasticity, excitatory/inhibitory imbalance, and disrupted neuronal synchrony

Synapsopathy in ASDs
ASDs: impaired neuronal network performance

Kelleher and Bear, *Cell* 135, October 31, 2008
Neuroinflammation in ASDs

- Microglia and astroglia are activated in brain in autism

- Fragile X Syndrome astrocytes can institute neuronal phenotype, and wild type astrocytes can rescue Fragile X Syndrome phenotype

- Wild type microglia rescue Rett Syndrome phenotype

Cytokines in ASDs

- Cytokines are cell signalling molecules produced by immune system cells including microglia

- Interleukin-6 is an example:
  - Interleukin-6 may be involved in autism, Fragile X Syndrome and Rett Syndrome
  - Interleukin-6 can activate microglia
  - IL-6 induces changes in dendritic spine density, alters neuronal adhesion and migration, causes excess in excitatory synapses, and reduces social interaction in an animal model of autism

Wei et al (2011) J Neuroinflammation 8:52
Glia-neuron interactions

Neuronal Transcriptional Control, Growth and Survival: Cytokines and Growth Factors in Fragile X Syndrome – Effect of NNZ-2566

NNZ-2566
Believed to attenuate aberrant cytokine levels

Activated Microglia
Believed to restore normal microglia function; perhaps through normalizing autocrine homeostasis

NNZ-2566
Reverses Akt in fmr1 KO mice

NNZ-2566
Reverses ERK in fmr1 KO mice

Growth Factors (BDNF, EGF)

Protein Synthesis Regulation
Normalised Dendritic Growth

Normal Dendritic Pruning
Cell Death / Apoptosis
Neuronal Transcriptional Control, Growth and Survival: Cytokines and Growth Factors in Rett Syndrome – Effect of NNZ-2566

NNZ-2566 Believed to attenuate aberrant cytokine levels
IGF-1(1-3) reported to increase IGF-1 receptor activation; may help reverse impaired Akt/mTOR activation in absence of MeCP2

Restoration of Microglia
NNZ-2566
Hypothesized to restore normal function of microglia. Increased microglial Atf3 is reported, this would reduce local IL-6 and TNF-α, attenuating autocrine activation. IGF-1(1-3) reported to activate Akt in microglia

Increase in total brain pAkt reported mice following IGF-1(1-3)

Protein Synthesis Regulation
Improved Dendritic Growth

Improved Dendritic Pruning
Cell Death / Apoptosis

Growth Factors (BDNF, EGF)

Caspase-3, Caspase-8, Bid, Bax

PI3K
Akt
mTOR

STAT3
NF-κB
Bad

MEK
ERK

Restoration of Microglia

NNZ-2566

TNF-α
IL-6
GP130
JAK3

Hypothesized to restore normal function of microglia.
Increased microglial Atf3 is reported, this would reduce local IL-6 and TNF-α, attenuating autocrine activation.
IGF-1(1-3) reported to activate Akt in microglia

Increase in total brain pAkt reported mice following IGF-1(1-3)
Mechanism of Action Summary

- NNZ-2566 is not a direct agonist of the IGF-1 receptor

- **Traumatic brain injury**
  - Microglial activation and cytokine release stimulates pro-apoptotic pathways
  - NNZ-2566 reduces neuroinflammation and apoptosis

- **Fragile X Syndrome**
  - Activation of astrocytes induces Fragile X Syndrome neuronal phenotype, potentially via induction of RAS-MEK-ERK and PI3K-Akt-mToR pathways
  - NNZ-2566 reverses neuroinflammation and Akt / ERK activation

- **Rett Syndrome**
  - Impaired passive sentinel function of microglia related to PI3k-Akt-mToR function
  - NNZ-2566 reverses Akt dysfunction normalizing microglial support of synaptic function
Effects on cytokines in brain injury
Time - Course of Gene Expression (qRT-PCR)

**IL-1β**
- Sham
- PBBI
- PBBI+NNZ-2566

**TNF-α**
- Sham
- 1hr
- 4hr
- 12hr
- 24hr
- 72hr
- 7ds

**IL-6**
- Sham
- 1hr
- 4hr
- 12hr
- 24hr
- 72hr
- 7ds

**E-selectin**
- Sham
- 1hr
- 4hr
- 12hr
- 24hr
- 72hr
- 7ds

Time Post-PBBI
Effects in Fragile X Syndrome model
Rescue of dendritic spines

- *fmr1* KO mouse neurons show increased numbers of dendritic spines
- NNZ-2566 in the concentration range 0.5 to 50 nM dose dependently reduces excess spine number
Open Field

The open field is an exposed space in which movement can be tracked. During exposure to the open field mice will habituate to the environment and thus explore less, decreasing the amount movement they show over time.

The present experiment records movement and rearing during an initial exposure, during a second exposure after 10 minutes and during a third exposure after 24 hours. Failures to reduce locomotion or rearing at 10 minutes and 24 hours indicate deficits in short and long term memory, respectively.

A variety of measures can be collected in the open field – locomotor activity and rearing are commonly reported.
Open Field – Rearing

- *fmr1* KO mice show increased activity
- NNZ-2566 has no effect in Wild Type mice, but significantly reduces rears in *fmr1* KO mice
- *fmr1* KO mice do not show habituation at 10 min (short term memory deficit)
- Thus during the short term and long term memory tests, NNZ-2566 treated *fmr1* KO mice do not differ from controls
Elevated Plus Maze – Time Spent

The elevated plus maze has two open and two closed arm, raised above floor height. The open arms are more exposed and therefore create more anxiety in the mice. Mice therefore spend more time in the closed arms and visit them more.

Measures taken include time spent in the arms and center of the maze, and number of arm entries.
Elevated Plus Maze – Time Spent

For time spent in both “Open” and “Closed” arm, *fmr1* KO mice show a significant difference to Wild Type controls, as well as time spent in the center.

This reflects hyperactivity in the *fmr1* KO mice, which spend much more time transiting the maze, compared to Wild Type mice, who remain in the closed arms.

This effect is significantly reversed following treatment with NNZ-2566.

*N = 10 per group*
Social Behavior – Bouts of Sniffing

- *fmr1* KO mice engage in significantly more bouts of sniffing compared to Wild Type mice
- NNZ-2566 has no significant effect in Wild Type mice
- After NNZ-2566 administration to *fmr1* KO mice, this group is not significantly different to NNZ-2566 Wild Type mice i.e. NNZ-2566 normalizes the abnormalities in social behavior seen in *fmr1* KO mice

N = 10 per group
Macro-orchididism

- *fmr1* KO mice show an increase in testis weight, as do human Fragile X Syndrome patients
- NNZ-2566 has no significant effect in Wild Type mice
- After NNZ-2566 administration to *fmr1* KO mice, testis weight is not significantly different from controls
Ras-Mek-Erk Pathway

- Overall levels of ERK are unchanged in the brain in fmr1 KO mice
- However, activation of ERK, as indexed by levels of phosphorylated ERK, is increased
- This activation is normalized by treatment with NNZ-2566
- NNZ-2566 may also increase total ERK levels in fmr1 KO mice

N = 4 per Group
Akt-Pathway

- Activation of Akt, as indexed by levels of phosphorylation, is increased
- This activation is normalized by treatment with NNZ-2566
Effects in Rett Syndrome model
Pharmacological Reversal of Phenotype

- IGF-1[1-3] reverses phenotype MECP2 mouse

Tropea et al. 2009
NNZ-2566 Rett Syndrome / Fragile X Syndrome

- Recruitment into Phase II clinical trial in Rett Syndrome is underway
  - 48 subjects; 2 doses plus placebo; 14 day follow-up
  - Adolescents and adults
  - Subjects will have baseline assessments of EEG, cardiac and respiratory rhythms, and functional status
  - Focus on safety and signals of efficacy

- Large market opportunity
  - Significant unmet need; no marketed therapeutic
  - Symptoms amenable to treatment
  - Potential for disease modification?
  - Potential “gateway” to autism

- Fragile X Syndrome study planning underway
Summary

- NNZ-2566 shows efficacy in pre-clinical models of:
  - Traumatic brain injury
  - Fragile X Syndrome
  - Rett Syndrome

- Efficacy accompanied by:
  - Decrease in microglial activation
  - Decrease in production of inflammatory cytokines
  - Normalization of Akt and ERK activation profiles

- Clinical studies now underway