Impact of Rett Syndrome on Patients' Lives

- Epilepsy: seizures vs Rett spells (episodes of autonomic dysfunction)
- GI Issues: feeding issues, GERD, constipation, abdominal distention/gas
- Growth and Nutrition: underweight
- Movement disorders: dystonia, chorea, tremor
- Autonomic dysfunction: breath-holding, hyperventilation, Rett spells
- Scoliosis/Kyphosis: impaired gait, impact on respiration
- Bone Health: frequent fractures due to low bone density
- Cardiac Issues: risk of QT prolongation
- Sleep Difficulties: sleep initiation, sleep maintenance
- Need for Therapies: PT, OT, and Speech Therapy

Medical management of Rett syndrome

Epilepsy (Tarquinio et al., Brain 2017)

- Most girls with Rett have abnormalities on baseline EEG by age 2
- Roughly 60-90 % of those with either typical or atypical Rett will have at least one seizure.
- Age of onset of epilepsy: 25% by age 3, 50% by age 6, and 75% by age 13
- Those with atypical Rett and a severe clinical phenotype had the highest prevalence of epilepsy.
- No particular anti-epileptic medication is more effective in Rett syndrome.
- Clinical features associated with increased seizure prevalence include:
  - frequency of hospitalizations, inability to walk, bradykinesia, scoliosis, gastrostomy feeding, age of seizure onset, and late age of diagnosis

Medical management of Rett syndrome

GI issues (Motil et al., J Pediatric Gastroenterology Nutr 2012):

- Gastrointestinal dysmotility: 92%
- Feeding difficulties: 81%
- Constipation: 80%
- Chewing dysfunction: 56%
- Gas bloating: 51%
- Swallowing dysfunction: 43%
- Gastroesophageal reflux: 38%
- Delayed gastric emptying: 14%
- Biliary tract disease: 4%

- Of note, some prokinetic agents like erythromycin may prolong the QTc, so these agents should be avoided in Rett patients with prolonged QT intervals.
  - Cyproheptadine used more commonly.

Medical management of Rett syndrome

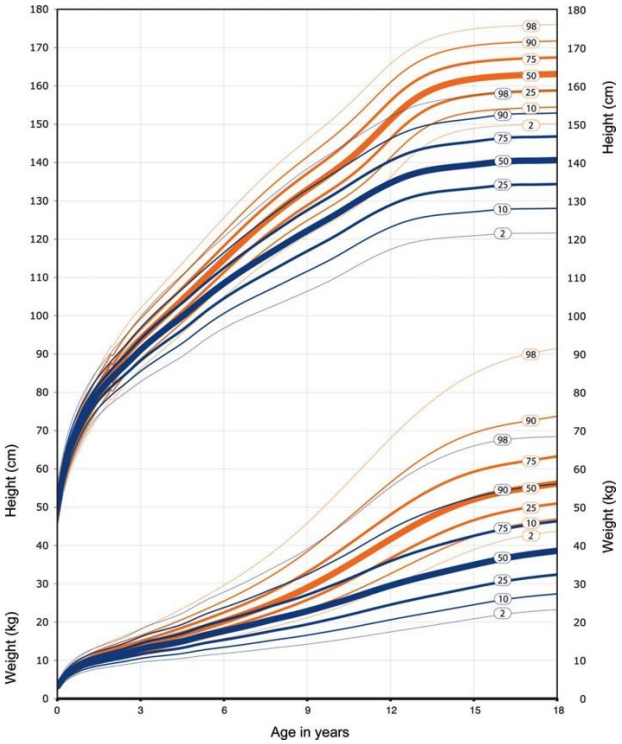
Growth and Nutrition

- Poor growth and weight gain are common problems in Rett syndrome.
- There are Rett specific growth curves we follow in clinic (next slide). No pubertal growth spurt seen.
- May initiate puberty earlier (adrenarche/thelarche i.e. Tanner 2 reached at 6-7 years in Rett vs 9 years in general population), but have menarche later (median age 13 years vs 12.5 years in general population).
- A body mass index of approximately the 25th percentile can be considered as a reasonable target in clinical practice.
- Gastrostomy (needed in 1/3 of patients) is indicated for extremely poor growth, to augment fluid intake, if there is risk of aspiration (dysfunctional swallow) and/or if feeding times are prolonged.

Medical management of Rett syndrome

Growth and Nutrition

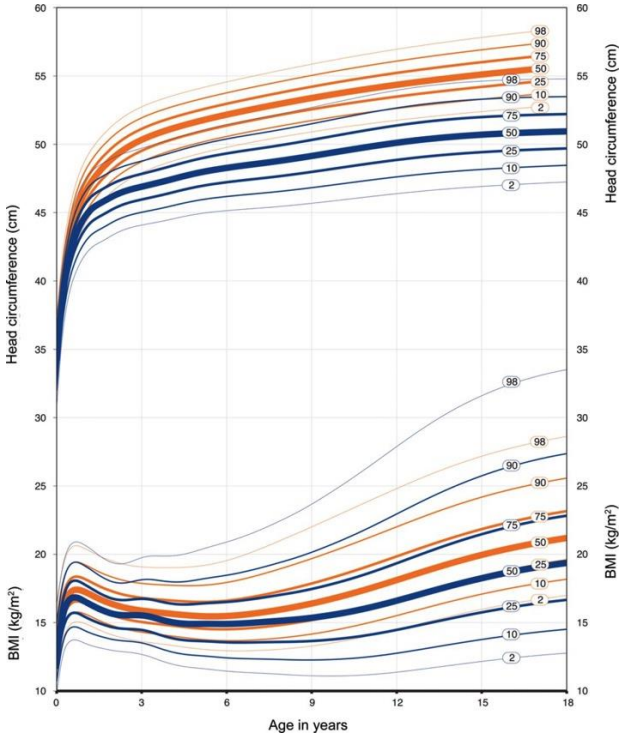
Height and Weight in unaffected children (orange) and children with classic Rett syndrome (blue)



Height

Weight

Head Circumference and Body Mass Index (BMI) in unaffected children (orange) and children with classic Rett syndrome (blue)



Head Circumference

BMI

Tarquinio DC1, Motil KJ, Hou W, Lee HS, Glaze DG, Skinner SA, Neul JL, Annese F, McNair L, Barrish JO, Geerts SP, Lane JB, Percy AK. Growth failure and outcome in Rett syndrome: specific growth references. Neurology. 2012 Oct 16;79(16):1653-61.

Medical management of Rett syndrome

Movement Disorders

- Hand stereotypies: bilateral hand wringing (75%), hand mouthing (35%), hand clasping/tapping (30%)
- Dyspraxic Gait: inability to plan and execute a voluntary motor sequence due to problems with muscle control and coordination
  - Inability is not due to weakness or involuntary muscle activity
  - Gait often broad based, short steps, may involve rocking/swaying
- Dystonia, tremor, myoclonus, chorea and athetosis
  - Dystonia (60%ile): contraction of both agonist and antagonist muscles. Cervical dystonia and limb dystonia. Can also lead to dystonic tremor.
  - Parkinsonism (40%ile): move slower, taking shorter steps, become more rigid, less variation in facial expression (hypomimia)
  - Myoclonus (35%ile)
  - Tremor (33%ile)
  - Chorea/Athetosis (15%ile)

Brunetti S and Lumsden D. Eur J Paediatr Neurol. 2020; 28:29-37; Hagberg B et al. Ann Neurol. 1983; 14(4):471-9; Fitzgerald PM et al. Neurology. 1990; 40(2):293-5; Temudo T et al. Neurology. 2007;68(15):1183-7.

Medical management of Rett syndrome

Autonomic Dysfunction

- **Breathing disturbances**:
  - More pronounced during wakefulness; but irregular breathing also occurs during sleep
  - Daytime alternating bouts of hypoventilation, irregular hyperventilation, and air swallowing
  - Mouse models of RTT suggests that different areas in the ventrolateral medulla and pons give rise to different aspects of this breathing disorder
  - Breathing regulation may be improved by serotonergic agents such as buspirone or SSRIs (sertraline, escitalopram, citolapram) or carbonic anhydrase inhibitors like acetazolamide and topiramate

- **Peripheral vasomotor disturbance**:
  - Hands and feet may be red, bluish, mottled, and cool, cold or hot to the touch due to variable autonomic influence of the smooth muscle tone around blood vessels

Julu PO et al. Arch Dis Child. 2001;85:29-37.

Medical management of Rett syndrome

Scoliosis (Downs et al., Spine 2016)

- Scoliosis is the most common orthopedic comorbidity in Rett syndrome seen in roughly 75% of individuals.
- The median age of scoliosis onset is 11 and it is progressive for nearly all mutation types (80% have significant scoliosis by age 16).
- Scoliosis progression was reduced when there was capacity to walk independently or with assistance.
- A Cobb angle of 40^{o} prior to the age of 12 years indicated that progression to a very severe scoliosis by age 16 was likely.
- Surgery should be considered when the Cobb angle is approximately 40^{o} to 50^{o}
- Durable Medical Equipment (DME): back/ankle/wrist braces, splints, wheelchair, walker, stander, bathing chair

Downs J et al. Spine. 2016;41(10):856-63.

Medical management of Rett syndrome

Bone Health

- More than 85% of patients develop osteopenia without treatment
- A baseline densitometry assessment (DEXA-Scan) should be performed
  - Decreased bone formation instead of increased bone resorption results in deficits in bone mineral mass in Rett syndrome
- Lateral spine x-rays are also suggested.
- Increasing physical activity and initiating calcium and vitamin D supplementation when low are the first approaches to optimizing bone health in Rett syndrome to enhance bone mineral deposition and reduce the frequency of bone fractures.
- If individuals with Rett syndrome meet the ISCD (International Society for Clinical Densitometry) criterion for osteoporosis in children, the use of bisphosphonates may be appropriate.

Haas RH et al. J Pediatr. 1997;131(5):771-4.

Medical management of Rett syndrome

Cardiac Issues

- Roughly 40% of girls with Rett syndrome have a prolonged QTc on EKG
- Prolonged QT syndrome is a serious disorder that can increase the risk of a potentially fatal ventricular arrhythmia (Torsades de Pointes) that may contribute to the increased risk of sudden death in Rett patients
- Yearly EKG's are recommended in all patients with Rett syndrome to identify the presence of QTc prolongation
- Medications that might cause QTc prolongation such as some antipsychotics, tricyclic antidepressants, etc. should be avoided in patients with a QTc abnormality

Crosson J et al. Am J Med Genet A. 2017;173(6):1495-1501.

Medical management of Rett syndrome

Sleep Issues

- Difficulty initiating sleep – 30% have trouble falling asleep more than once/week
- Difficulty maintaining sleep – 50% have night awakenings more than once/week
- Poor sleep contributes to daytime somnolence, increased seizure frequency, behavior dysregulation, and decreases sleep duration of family members
- Consider contribution of seizures, GI reflux, pain related to constipation or muscle spasms to night time awakenings
- Need to reinforce good sleep hygiene
- Consider melatonin, clonidine, trazodone, mirtazapine, or gabapentin
  - Remember to check EKG for QTc prolongation with trazodone and mirtazapine

Boban S et al. *Am J Med Genet*. 2018;176(7):1569-1577. Glaze DG et al. *Ann Neurol*. 1987;21(4):377-82.

Long Term Prognosis

- With appropriate care, children with Rett syndrome will become adults with Rett syndrome, with over 70% of affected individuals living to at least 50 years of age

- The most frequently reported causes of death (one-quarter of deaths) are variations of sudden, unexplained death with no apparent underlying cause, likely due to uncontrolled seizures, fatal arrhythmia, aspiration, and pneumonia associated with lack of mobility.

- Most individuals with Rett syndrome require substantial assistance with every aspect of daily living.

- Over time, we often see:
  - Increase in motor problems
  - Decrease in irritability, and an improvement in eye contact, interaction, and communication.
  - Decrease in seizures and irregular breathing

- Primary Care Guidelines: www.rettsyndrome.org/for- families/education/primary-care-guidelines (Fu C, et al. BMJ Paediatr Open 2020 Sep13: 4)

Current management of Rett syndrome

- Epilepsy: Depakote, **Trileptal**, Zonisamide, Keppra
- GI Issues: **omeprazole**, **Miralax**, **Senna**, Milk of Magnesia, G-tube
- Growth and Nutrition: Duo-Cal, **Natural Harvest**, Nutren 2.0
- Movement disorders: Artane, Sinemet
- Autonomic dysfunction: **Sertraline**, Lexapro, Celexa, Prozac
- Scoliosis/Kyphosis/impaired gait: TLSO brace, AFO's
- Bone Health: **Vitamin D3, calcium carbonate**, zoledronic acid
- Cardiac Issues: Propranolol, Betaxolol
- Sleep Difficulties: **Clonidine**, Trazodone
- Physical Therapy: use of **gait trainer**
- Occupational Therapy: use of elbow brace
- Speech Therapy: Use of augmentative and alternative communication
  - Eye gaze based devices: **Tobii dynavox** and PRC Accent device

Current management of Rett syndrome

- Parents most interested in better treatments for:
  - Seizures and Rett Spells, communication, hand function, ambulation, sleep, and constipation
- How do we cut down on the number of medications and interventions used to treat and manage complications of Rett syndrome?
- How do we improve core features of Rett syndrome that current medications cannot treat?
- When do we have to intervene?
  - While symptoms typically appear postnatally, and the disease course is progressive, Rett syndrome is not a neurodegenerative disorder.
  - There is no pronounced death of neurons in Rett syndrome, but there is reduction in the size of the neuron, the extent of branching of the neurons, and the extent of connections between neurons.
  - Can we improve symptoms of Rett syndrome years after the diagnosis?

Treatment Landscape

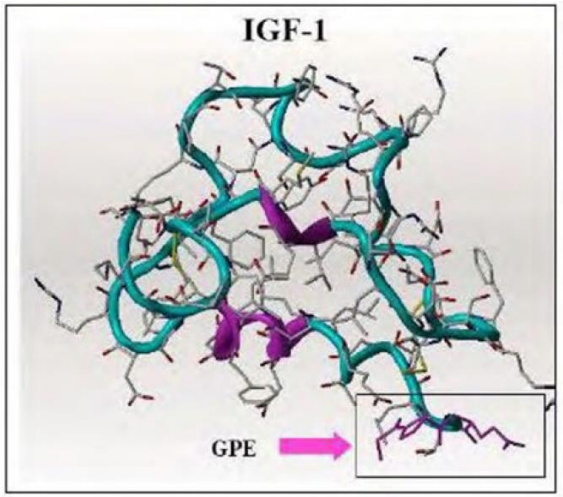
Pharmaceutical Compounds

There are no FDA approved treatment options for the management of Rett syndrome

- Current management of Rett syndrome is supportive, typically utilizing a single medication to treat a particular symptom
- We don't have medications that treat the ***core symptoms*** of Rett syndrome
    - No medication available to improve ambulation, hand function, or communication
- Medications that improve neuronal function in general have been trialed in Rett syndrome to measure impact on global function of the individual with RTT
- Phase 3 trials of two compounds show positive top-line results (data from manufacturer websites). Studies have not yet been peer reviewed.
- **Trofinetide**
- **Blacarmesine**

Trofinetide- Mechanism of Action

- Trofinetide (NNZ-2566): 2-methylproline-substituted analogue of glycine-proline-glutamate, which is the N-terminal tripeptide cleavage product of insulin-like growth factor 1 (IGF-1)
- Improves synaptic function and restore synaptic structure
- Inhibits overactivation of inflammatory microglia and astrocytes
- Increases the amount of IGF-1 in the brain
- Systemic treatment with the active tripeptide fragment of IGF-1 in Mecp2 knockout mice extends the lifespan of the mice, improves locomotor function, improves breathing patterns, reduces irregularity in heart rate, and partially restores spine density and synaptic amplitude.



Tropea D et al. *Proc Natl Acad Sci U S A*. 2009;106(6):2029-34.

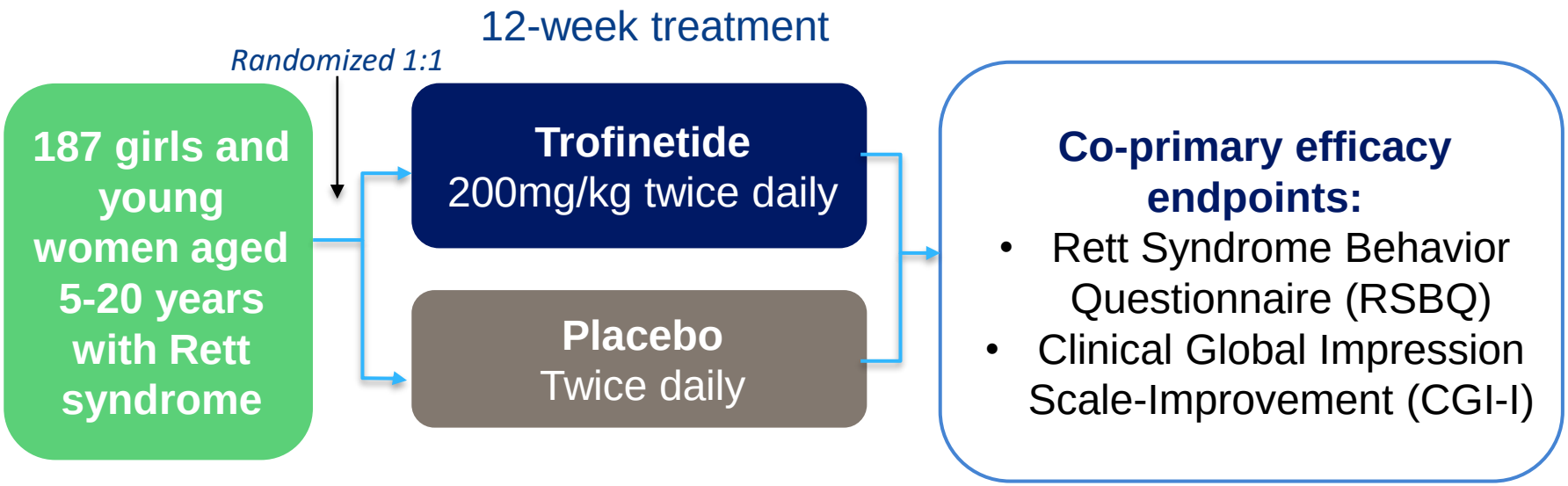
Trofinetide: Preliminary Trials

Trofinetide: Favorable Safety and Tolerability Profile in Initial Studies

- Neu-2566-RETT-001 (NCT01703533); phase II trial in females 16-45 years
  - Tested 35 mg/kg BID vs 70 mg/kg BID vs placebo[1]
- Neu-2566-RETT-002 (NCT02715115); phase II in trial in females 5-15 years
  - Trialed 50 mg/kg BID vs 100 mg/kg BID vs 200 mg/kg BID vs placebo[2]

[1]Glaze DG et al. *Pediatr Neurol.* 2017;76:37-46. [2]Glaze DG et al. *Neurology*. 2019;92(16):e1912-e1925.

Phase 3 LAVENDAR Trial



- Ongoing open-label Lilac extension study
- As of July 2022, A New Drug Application (NDA) for trofinetide has been submitted to the Food and Drug Administration (FDA) for the treatment of Rett syndrome in patients 2 years of age and older

Neul JL et al. *Contemp Clin Trials.* 2022;114:106704.

Trofinetide: *RSBQ and CGI-I*

Rett Syndrome Behavior Questionnaire (RSBQ):

- Validated 45 item rating scale, completed by the caregiver
- 8 general neurobehavioral areas specific to Rett
- Score: 0 (not true), 1 (sometimes true), 2 (often true)
- Has been correlated with functioning & quality of life in Rett
- Example: “Spells of inconsolable crying for no apparent reason during the night”

Clinical Global Impression of Improvement (CGI-I): Uses a 7-point scale

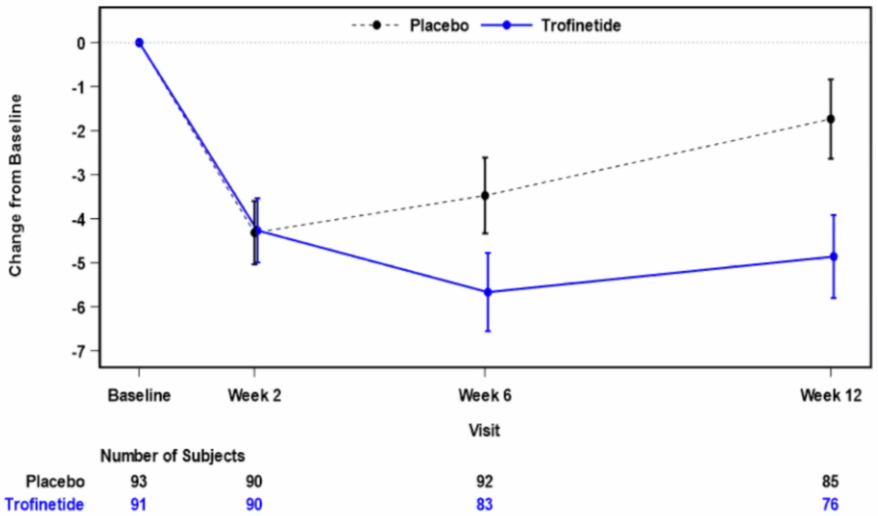
1 - Very Much Improved
2 - Much Improved
3 - Minimally Improved
4 - No Change
5 - Minimally Worse
6 - Much Worse
7 - Very Much Worse

Mount RH et al. *J Child Psychol Psyc.* 2002;43:1099-110.

Trofinetide

Coprimary Efficacy Endpoints

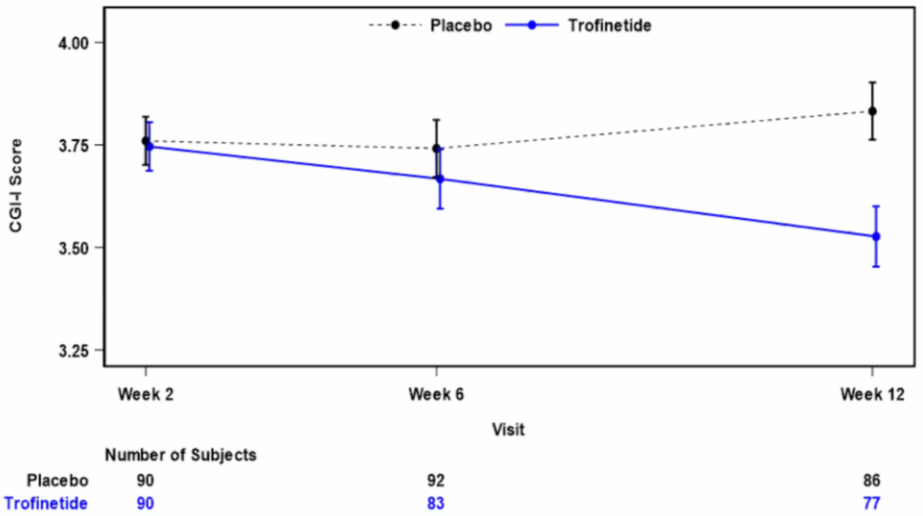
Rett Syndrome Behaviour Questionnaire (RSBQ)



Number of Subjects				
Placebo	93	90	92	85
Trofinetide	91	90	83	76

RSBQ change from baseline to week 12:
p-value = 0.0175*
Effect Size = 0.37

Clinical Global Impression – Improvement (CGI-I)



Number of Subjects			
Placebo	90	92	86
Trofinetide	90	83	77

CGI-I at Week 12:
p-value = 0.0030*
Effect Size = 0.47

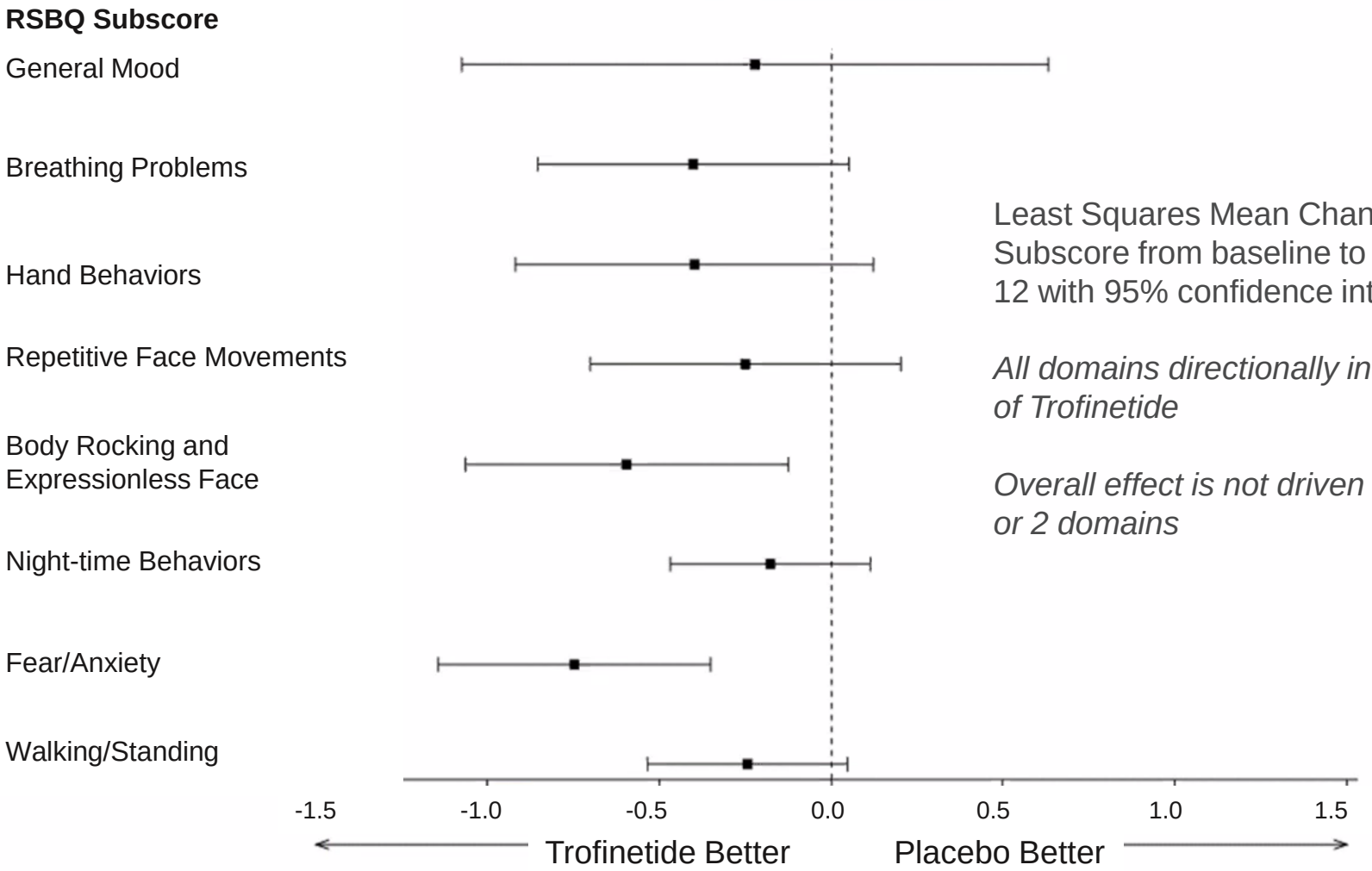
**p*-values based on least squares mean from the mixed-effects model for repeated measures analysis.

Statistically significant improvement over placebo in the RSBQ (p=0.0175) and the CGI-I (p=0.0030).

Lavender Study Positive Top-line Results for the Treatment of Rett Syndrome. (Investor Presentation December 6, 2021)
https://ir.acadia.com/static-files/84457c64-60ab-4b2f-a166-edc1d465f4a8

Trofinetide

RSBQ Subscores Treatment Differences



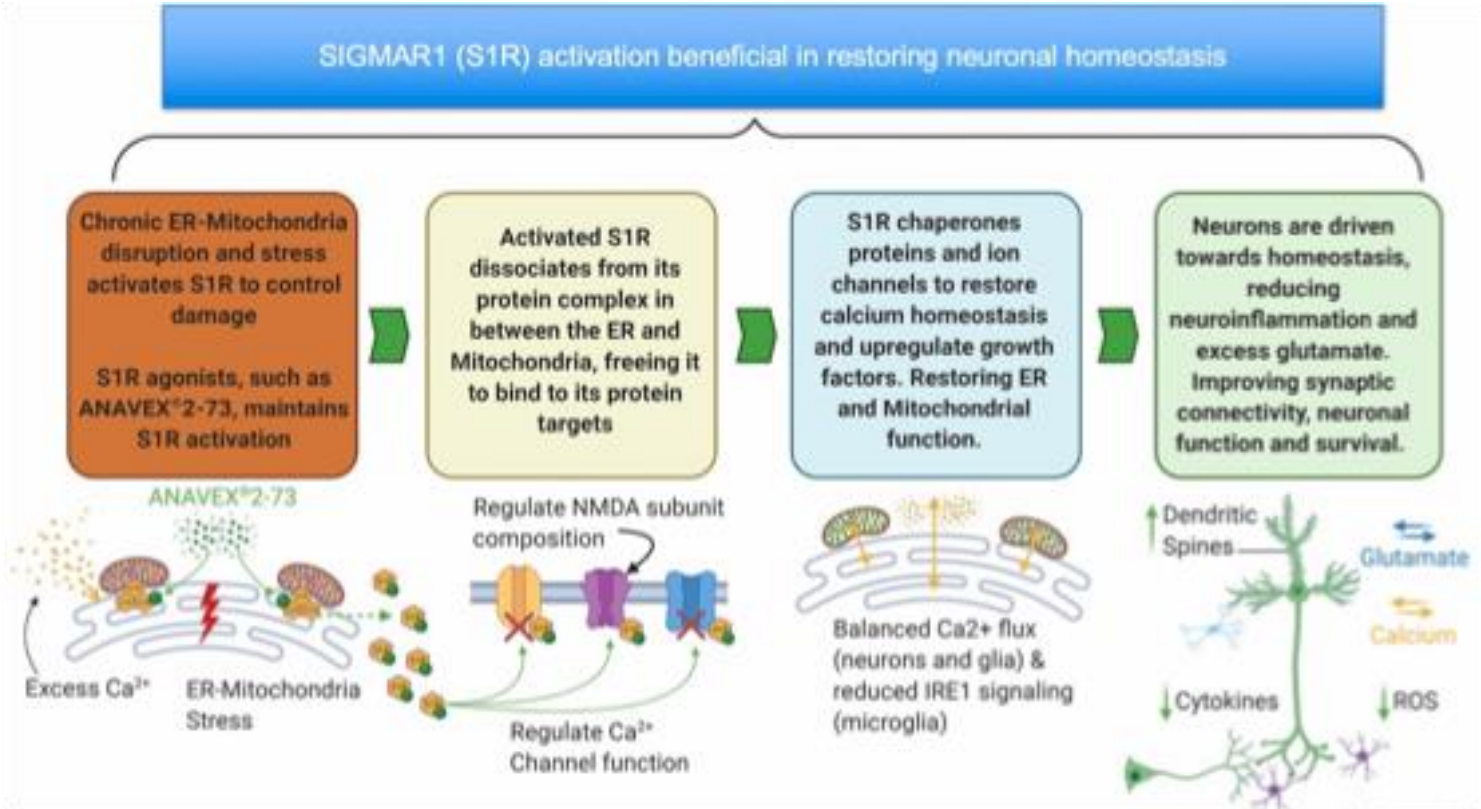
Least Squares Mean Change in Subscore from baseline to week 12 with 95% confidence interval

All domains directionally in favor of Trofinetide

Overall effect is not driven by 1 or 2 domains

Lavender Study Positive Top-line Results for the Treatment of Rett Syndrome. (Investor Presentation December 6, 2021)
https://ir.acadia.com/static-files/84457c64-60ab-4b2f-a166-edc1d465f4a8

Blacarmesine- Mechanism of Action



- Activates sigma-1 receptors in endoplasmic reticulum and modulates the activity of ion channels and signaling molecules, including inositol phosphates, protein kinases, and calcium channels to modulate calcium homeostasis and mitochondrial function.
- Activation of sigma-1 receptors can attenuate oxidative stress linked to inflammation and increase release of brain-derived neurotropic factor (BDNF), which is decreased in MECP2 deficiency.

Ryskamp DA et al. *Front Neurosci.* 2019;13:862.

Blacarmesine
Clinical Trials

- Phase 2 safety, tolerability, and efficacy study in Adults (NCT03758924): Completed
  - 31 patients, 18 to 45 years treated for 7 weeks
- AVATAR Phase 3 Adult Rett Syndrome Trial (NCT03941444): Completed
  - 33 participants, 18 years or older, treated for 7 weeks
- EXCELLENCE Phase 2/3 Pediatric Rett Syndrome Trial (NCT04304482): Ongoing
  - 84 participants, 5-17 years of age, treated for 12 weeks
  - Endpoint Assessments:
    - Primary: Assessment of RSBQ AUC response and safety
    - Secondary: Emotional behavior response (ADAMS) and CGI-I (Clinical Global Impression of Improvement)

Blacarmesine
ADAMS

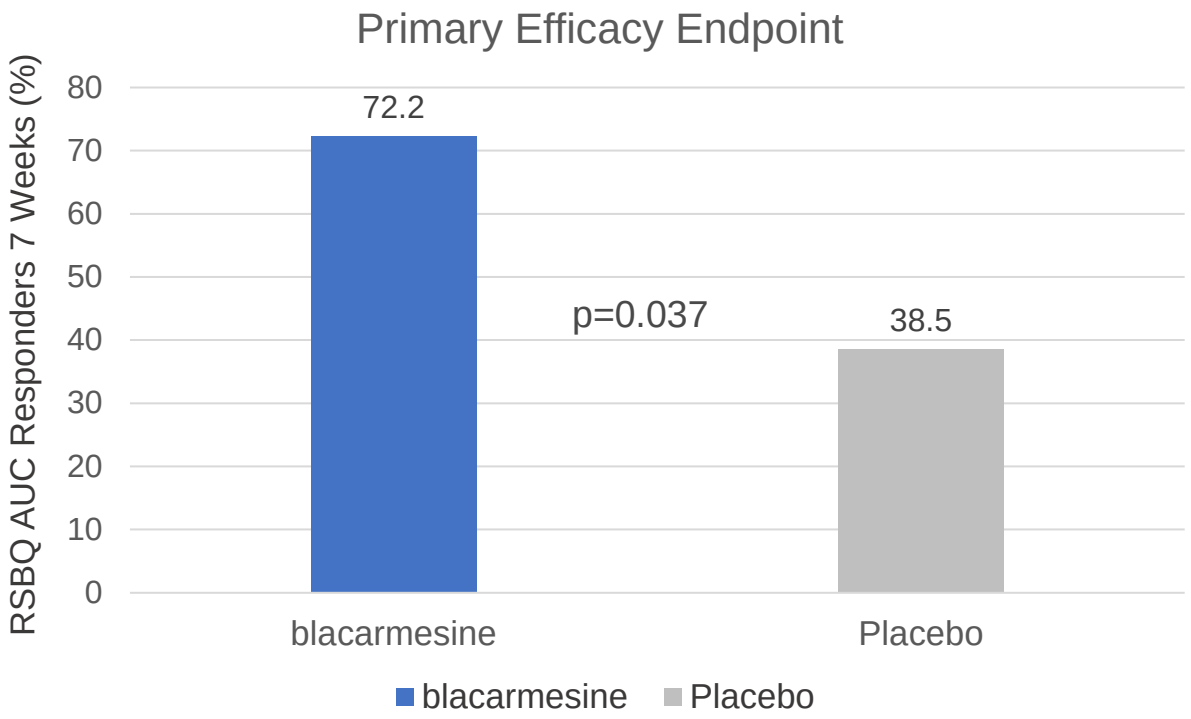
- The Anxiety, Depression, and Mood Scale (ADAMS) is a measure of anxiety and mood symptoms in individuals with intellectual disability which has been clinically validated for use in Rett syndrome

- The ADAMS generates a total score and 5 subscale scores:
    - Manic/hyperactive behavior
    - Depressed mood
    - Social avoidance
    - General anxiety
    - Obsessive compulsive behavior

Esbensen AJ, et al. *J Autism Dev Disord.* 2003;33(6):617-29

Blacarmesine
Primary Endpoint of AVATAR Study

RSBQ in those who showed at least a 1-point improvement in CGI-I score



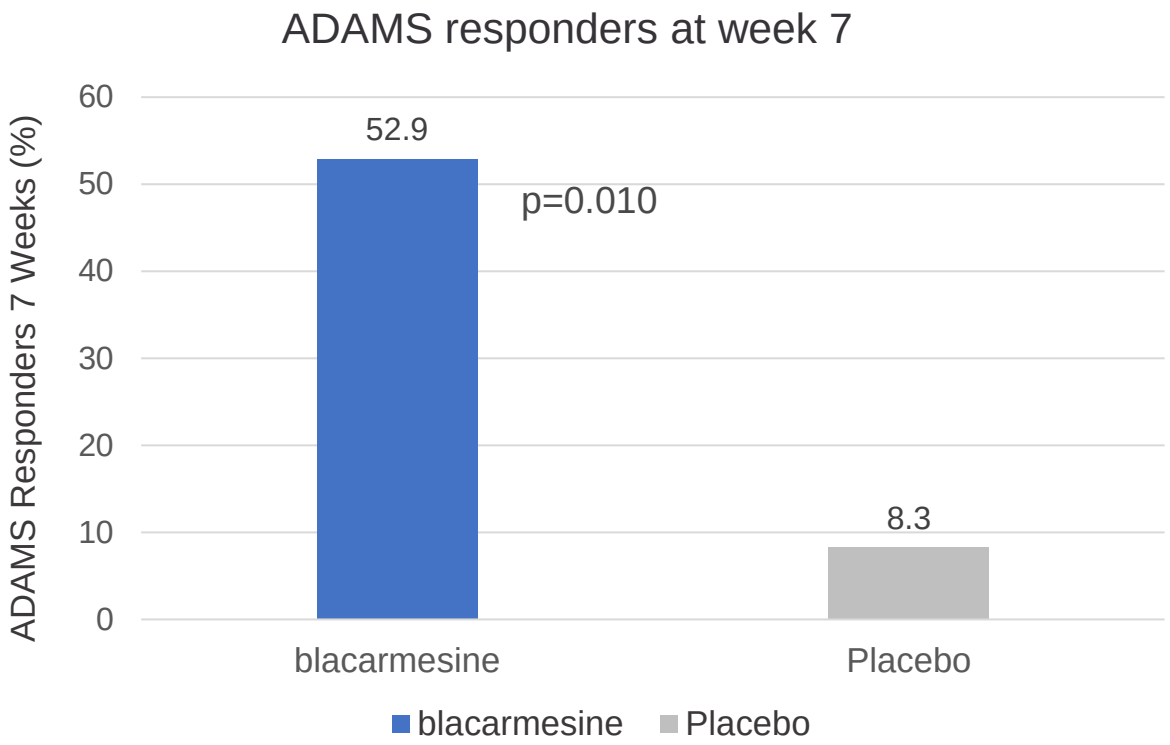
- Blacarmesine induces a clinical meaningful improvement of RSBQ AUC* in 72.2% of patients as compared to 38.5% on placebo; (p=0.037)
- Cohen's d effect size 1.91 (very large)

**Improvement threshold of at least 1 full point in the CGI-I scale from 'No Change' (i.e., 4) to at least 'Minimally Improved' (i.e., 3) or 'Much Improved' (i.e., 2) or 'Very Much Improved' (i.e., 1)*

Anavex press release: https://www.anavex.com/post/anavex-2-73-blarcamesine-avatar-phase-3-trial-met-primary-and-secondary-efficacy-endpoints
ClinicalTrials.gov Identifier: NCT03941444

Blacarmesine
Secondary Endpoints of AVATAR Study

ADAMS in those who showed at least 20% improvement in ADAMS score



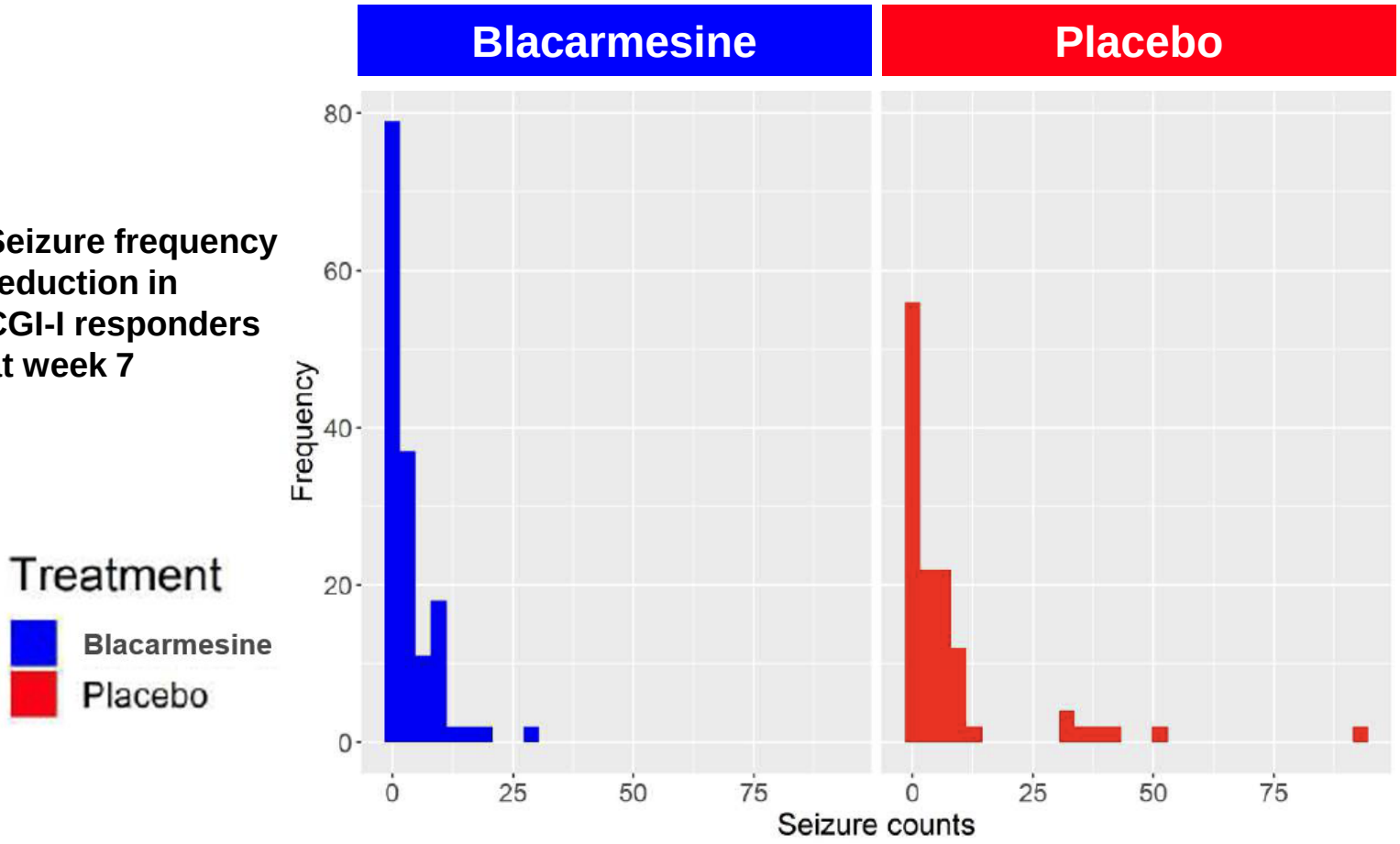
- Clinically meaningful and statistically significant reduction of emotional behavioral symptoms (ADAMS) response* for blacarmesine treated adult patients with Rett syndrome (52.9%) vs placebo (8.3%); (p=0.010)
- Cohen's d effect size 0.609 (large)

**Improvement threshold of at least -20% change (improvement) of ADAMS total score from baseline*

Blacarmasine press release: https://www.anavex.com/post/anavex-2-73-blarcamesine-avatar-phase-3-trial-met-primary-and-secondary-efficacy-endpoints
ClinicalTrials.gov Identifier: NCT03941444

Blacarmesine
Secondary Endpoints

Seizure frequency reduction in CGI-I responders at week 7

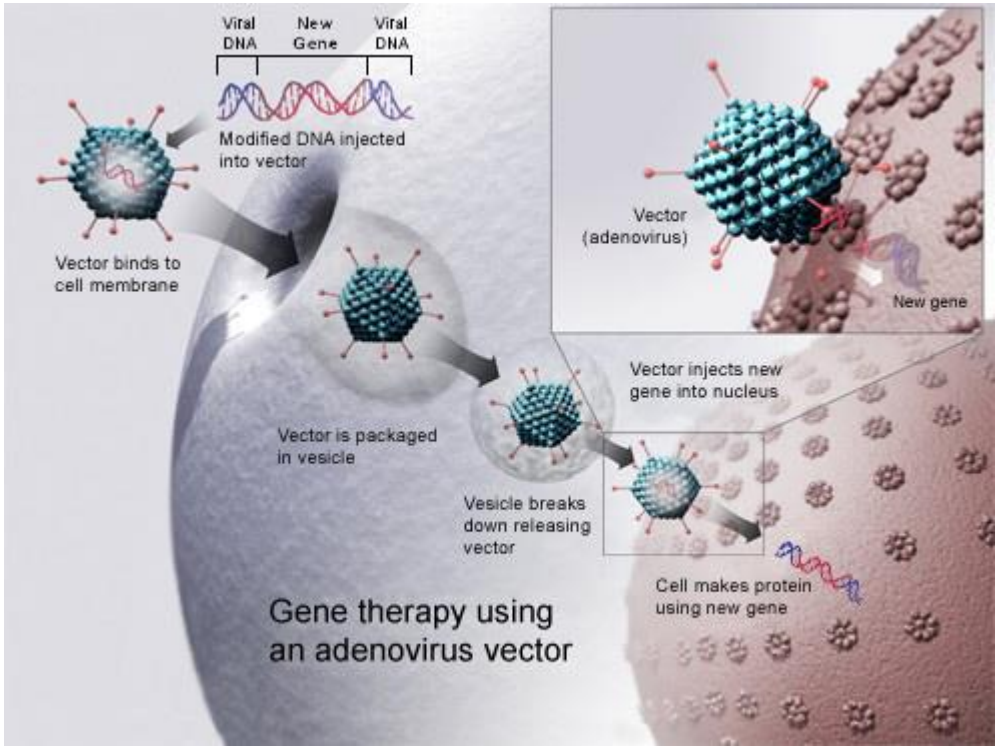


- Blacarmesine is associated with a 50.7% reduction in weekly seizure risk

Blacarmasine press release: https://www.anavex.com/post/anavex-2-73-blarcamesine-avatar-phase-3-trial-met-primary-and-secondary-efficacy-endpoints
ClinicalTrials.gov Identifier: NCT03941444

Gene Therapy

Goal: Introduce a full functional copy of MECP2 into the brain of individuals with Rett syndrome



Phase 1/2 open-label trials to evaluate safety, tolerability, and efficacy using an AAV9 virus to introduce MECP2 into neurons deficient in functional MECP2

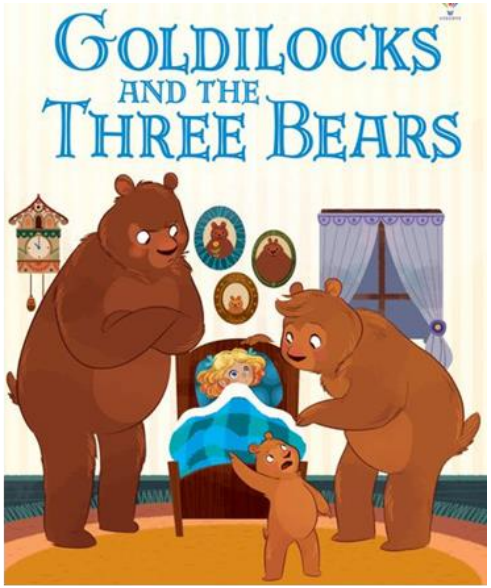
- **TSHA-102**
- **NGN-401**

MECP2 is a dosage sensitive gene, with both animal studies and the human *MECP2* Duplication Syndrome suggesting that *MECP2* levels need to be kept within a narrow range to achieve efficacy while avoiding overexpression related toxicity.

Sinnett SE et al. *Brain.* 2021;144(10):3005-3019. Cobb S. *A Self-regulating Gene Therapy for Rett Syndrome*. Abstract; ASGCT; May 2022.

Gene Therapy

- Overexpression of the *MECP2* gene can lead to symptoms of MECP2 Duplication Syndrome:
  - intellectual disability
  - limited to absent speech
  - infantile axial hypotonia that often leads to progressive spasticity
  - movement disorders (including chorea and tremor)
  - susceptibility to respiratory infections
  - autistic features
  - difficult-to-control epilepsy
- Too little protein could result in perhaps a milder form of Rett syndrome phenotype



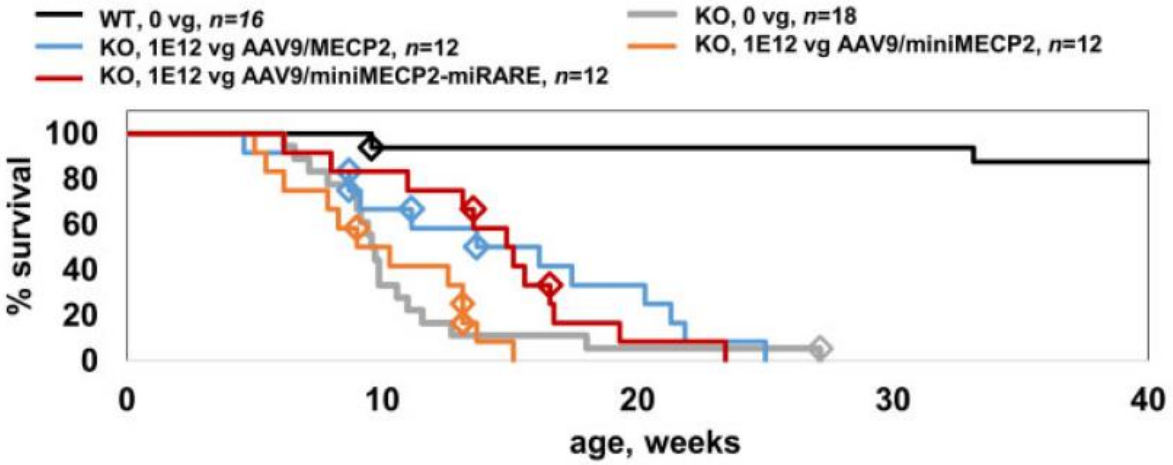
The amount of *MECP2* protein:

- Can't be too little
- Can't be too much
- Needs to be just right

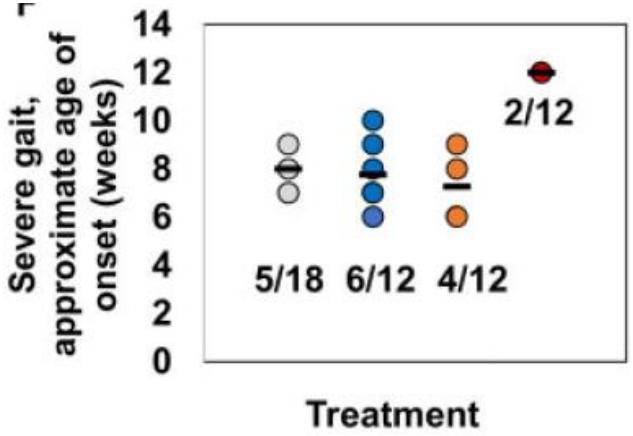
D'Mello SR 3rd. *J Neurochem.* 2021;159(1):29-60.

Gene Therapy: TSHA-102

- AAV9/miniMECP2-miRARE (microRNA responsive auto-regulatory element, ‘miRARE’ to minimize the possibility of miniMECP2 transgene overexpression) mice live longer and show fewer phenotypic deficits including a functional measure like ambulation



Median survival for knockout mouse given saline (gray line: 9.6 weeks) versus AAV9/miniMECP2-miRARE-treated knockout mouse (red line: 15 week; p< 0.02).



Frequency of achieving severe gait (score = 2): saline (saline: 28%), AAV9/MECP2 (50%), AAV9/miniMECP2 (33%) and miRARE-treated mice (red line: 17%).

Sinnett SE et al. *Brain.* 2021;144(10):3005-3019.

Gene Therapy: NGN-401

- Transgene expression is regulated by a miRNA-based feedforward loop (termed EXACT)
  - A cell autonomous mechanism to prevent overexpression while still allowing expression of therapeutic protein levels
- **Not based on any existing mammalian miRNA**, preventing interference with endogenous gene regulation in transduced cells
- Using unregulated constructs in the *Mecp2* null mouse, *MeCP2* levels increased proportionally with plasmid dose, while for regulated constructs, protein levels displayed relative dosage insensitivity and were maintained within a much narrower range or setpoint.
- Mecp2 knockout mice treated with the regulated lead construct showed a profound improvement in lifespan (median survival increased from 12 to 35 weeks) and significant amelioration of RTT-like phenotypes.
- Using the lead construct in non-human primates, early in-life safety was demonstrated in separate toxicity studies.

Cobb S. *A Self-regulating Gene Therapy for Rett Syndrome*. Abstract; ASGCT; May 2022.

Key Takeaways

- Rett syndrome is a life-long neurodevelopmental disorder that affects an individual's gross motor, fine motor, and communication skills with co-morbid problems stemming from epilepsy, gastrointestinal dysfunction, nutritional deficits, movement disorders, autonomic dysfunction, orthopedic issues, cardiac concerns, and mood regulation difficulties

- Despite these widespread issues, girls, boys, and women with Rett syndrome can participate in life at home, at school, and in their community and can be expected to live into their 50s, 60s and 70s

- Two emerging targeted therapies may improve quality of life for individuals with Rett syndrome and will soon be presented to the FDA for possible approval

- Gene Therapy trials have launched and may provide meaningful improvements in the lives of our patients and their families

Thank You

- To our patients.
- To our families.
- To the Rett Syndrome Association of Massachusetts (RSAM, now Rett Syndrome Angels)
- To the International Rett Syndrome Foundation and the Rett Syndrome Research Trust for their support

International Rett Syndrome Foundation
www.rettsyndrome.org

rett syndrome research trust
Making Rett History