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Profile of Trofinetide in the Treatment of Rett Syndrome: Design, Development and Potential Place in Therapy

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Abstract: Trofinetide is a first-in-class pharmacological treatment proposed for patients with Rett Syndrome. It is a long half-life derivative of glycine-proline-glutamate, the tripeptide normally excided from Insulin-like Growth Factor 1 upon degradation. Due to containing glutamate and glycine in its structure, trofinetide is thought to act through NMDA receptor modulation, thus providing a normalization of neuronal activity and survival. Trofinetide was tested in a series of short and long-term trials, showing good efficacy at improving scores on the Clinical Global Impression-Improvement scale and Rett Syndrome Behavior Questionnaire, with specific effect only on some subscales, ie General Mood subscale and Repetitive Face Movement subscale. No effects were documented on other subscales or on epilepsy, heart and bone –related symptoms. The main adverse effects of trofinetide, severe enough to determine discontinuation, include diarrhea, vomiting, and consequent weight loss. These may be scarcely avoidable, given the need to assume a very large amount of trofinetide per day. Other inherent limitations of use possibly regard the limited duration of drug supplies, as one bottle may last three days only, depending on weight, and the relatively high cost per bottle. Trofinetide has no direct competitors: single symptoms of the Rett Syndrome, for instance, seizures or aggressive behaviors, are currently treated with drugs that have been developed for patients without the Rett Syndrome. This leads to suboptimal efficacy and increased risk of adverse effects. The place in therapy of trofinetide is yet to be determined, based on the results of clinical trials, on its practical usability, and on the windows of opportunity for intervention. Moreover, trofinetide may be curative if given early enough during brain development, or merely symptomatic if given to young adults, and no data exist on this aspect. The place in therapy of trofinetide will require reassessment after competing treatments enter the market.

Keywords: IGF-1, GPE, GH, NMDA, efficacy, safety

Introduction

Trofinetide is the first drug approved specifically for the treatment of patients with Rett Syndrome. It is a long-acting derivative of glycine-proline-glutamate (GPE), the tripeptide generated by enzymatic cleavage of Insulin-like Growth Factor 1 (IGF-1). To date, several reviews are available that deal with the chemical and preclinical development of this compound.[1,2] However, the literature is lacking with respect to defining the clinical needs of patients affected by the Rett Syndrome and there is no review available on the current therapeutic options. Therefore, in this narrative review, we will provide an overview of the clinical picture and the therapeutic alternatives already available for clinicians. This will allow us to focus on the place in therapy of trofinetide, with respect to the complex symptoms of the Rett Syndrome and the heterogeneous picture of concomitant drug treatments in routine clinical use.

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Materials and Methods

The initial literature analysis was performed using the Epistemic AI platform[3] using the results obtained by searching what drugs are used and being developed for the Rett Syndrome.

The narrative literature review was conducted in PubMed database using the search terms (Rett syndrome) (treatment) (drug); this search resulted in 489 articles. Inclusion criteria were being published in the last 20 years (444 articles retained), being conducted on human populations (297 articles retained) and written in English (280 articles retained). We screened articles and excluded all those who were not clinical studies, clinical trials, randomized controlled trials or meta-analysis. Finally, we analyzed the subset of 38 selected articles. After reading the full texts, 12 articles were selected based on the relevance of the studied drug treatments.

Rett Syndrome

Rett Syndrome (RTT; OMIM entry #312750) is a genetic neurodevelopmental disorder predominantly affecting females, with a prevalence ranging from 1:10,000 to 1:23,000 female live births. Though RTT diagnosis is mainly clinical, more than 95% of cases are caused by mutations in the gene encoding the methyl-CpG binding protein 2 (*MECP2*), a transcriptional regulator involved in chromatin remodeling and the modulation of RNA splicing.[16,17] The variation spectrum of *MECP2* mutations appears to be independent of ethnicity.[18]

RTT Presentation

RTT is characterized by a unique developmental regression period, occurring after apparently normal early development, with the onset of autistic-like features including intense stereotypic midline hand movements, impairment of communicative abilities and of fine motor skills.[19] Other clinical features include postnatal deceleration of head growth, epilepsy, cognitive impairment, scoliosis, feeding difficulties, growth restriction, sleep disturbance, bruxism, autonomic and motor dysfunction. RTT diagnosis is clinically based on distinct criteria revised in 2010, independent of molecular findings.[19] With respect to previous criteria of 2002, the revision recommended the presence of regression plus four main criteria to be required for the diagnosis of typical RTT. The four main criteria included:

1. Partial or complete loss of acquired purposeful hand skills;
2. Partial or complete loss of acquired spoken language;
3. Gait abnormalities: impairment (dyspraxia) or absence of ability;
4. Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing and washing/rubbing automatisms.

The post-natal deceleration in head growth was eliminated from the necessary criteria because this feature is not found in all individuals with typical RTT.[19]

RTT Staging

The symptoms evolve with development and typically progress through four stages:[17,19,20]

Stage 1 is known as the "early stagnation stage" (6–18 months). During this phase, the symptoms may be very subtle at first and may go unnoticed. The main symptoms are issues with sitting, crawling, and walking, lack of interest in toys, problems in speech development, low muscle tone, or hypotonia.

Stage 2 is known as the "rapid destructive phase" (1–4 years) characterized by loss of previously acquired motor, communication, and social skills, and loss of the ability to use hands purposefully. Instead, patients show abnormal hand movements such as clasping or squeezing, clapping or tapping, and hand-washing movements, irritability characterized by children screaming or crying without reason, mobility and coordination problems including unsteady gait, reduced head circumference (acquired microcephaly), and emergence of breathing issues such as rapid breathing and breath holding.

Stage 3 is known as the "pseudostationary stage" or "plateau phase" that usually begins between ages 2 and 10 and can last for many years. During this phase, there may be improvements in hand usage, alertness, communication skills, walking ability, and irritability (less crying). The main symptoms of stage 3 are seizures, worsening of breathing problems, development of cardiac arrhythmias (abnormal heart rhythm) in some children, teeth grinding, difficulty to gain and maintain weight.

Stage 4 is known as the "late motor deterioration stage". This usually begins at about age 10 and can last for years or decades. The main symptoms during this phase are reduced mobility, muscle weakness and stiffness, stiffness in joints, scoliosis (abnormal curvature of the spine), and loss of walking ability.[21]

Rett Syndrome Behavior Questionnaire (RSBQ)

The RSBQ is a validated assessment tool that has been accepted by the FDA as a primary outcome measure for clinical studies on RTT.[22] It is a caregiver-rated scale that assesses neurological and behavioral symptoms in patients between 2 and 47 years of age. The RSBQ consists of 45 items (Table 1), 38 of which are divided into 8 subscales corresponding to the symptomatological domains of RTT such as general mood, breathing problems, hand behaviors, repetitive movements, night-

Table 1 RSBQ Questionnaire

Domains/ Subscales	Item	Max Score
General mood	• Spells of screaming for no apparent reason during the day • Abrupt changes in mood • Certain days/periods where she performs much worse than others • There are times when she appears miserable for no apparent reason • Screams hysterically for long periods of time and cannot be consoled • There are times when she is irritable for no apparent reason • Spells of inconsolable crying for no apparent reason during the day • Vocalizes for no apparent reason	16
Breathing problems	• There are times when breathing is deep and fast (hyperventilation) • There are times when breath is held • Air or saliva is expelled from mouth with force • Swallows air • Abdomen fills with air and sometimes feels hard	10
Hand behaviors	• Does not use hands for purposeful grasping • Hand movements are uniform and monotonous • Has frequent naps during the day • Restricted repertoire of hand movement • Has difficulty in breaking/stopping hand stereotypies • The amount of time spent looking at objects is longer than the time spent • holding or manipulating them	12
Repetitive face movements	• Makes repetitive movements involving fingers around tongue • Makes mouth grimaces • Makes repetitive tongue movements • Makes grimacing expressions with face	8
Body rocking and expressionless face	• Expressionless face • Seems to look through people into the distance • Uses eye gaze to convey feelings, needs, and wishes • Rocks self when hands are prevented from moving • Tendency to bring hands together in front of chin or chest • Rocks body repeatedly	12

(*Continued*)

Table 1 (Continued).

Domains/ Subscales	Item	Max Score
Nighttime behaviors	• Spells of screaming for no apparent reason during the night • Spells of laughter for no apparent reason during the night • Spells of inconsolable crying for no apparent reason during the night	6
Fear/Anxiety	• Spells of apparent anxiety/fear in unfamiliar situations • Seems frightened when there are sudden changes in own body position • There are times when parts of body are held rigid • Spells of apparent panic	8
Walking/ Standing	• Although can stand independently tends to lean on objects or people • Walks with stiff legs	4
Uncategorized	• Spells of laughter for no apparent reason during the day • Has wounds on hands as result of repetitive hand movements • Shifts gaze with slow horizontal turn of head • Makes repetitive hand movements apart • Appears isolated • Grinds teeth • Vacant "staring" spells	7

Note: Reproduced with permssion from Mount RH, Charman T, Hastings RP, Reilly S, Cass H. The Rett Syndrome Behaviour Questionnaire (RSBQ): refining the behavioural phenotype of Rett syndrome. J Child Psychol Psychiatry. 2002 Nov;43(8):1099-110. Copyright 2002, John Wiley and Sons.[24]

time behaviors, anxiety, and eye movements. Each item is rated on a Likert scale as 0 (behavior "not true"), 1 (behavior "somewhat or sometimes true") or 2 (behavior "often true"), with the total score ranging from 0 to 90, indicating the severity and frequency of symptoms. The RSBQ has been correlated with functioning and quality of life.[22,23]

RTT and Putative Mechanisms of Action

The typical RTT is determined by loss-of-function mutations of the methyl-CpG binding protein 2 (*MECP2*) gene. Other mutations of *MECP2* and/or dysregulations of its expression lead to atypical forms of RTT; moreover, other syndromes similar to RTT can be determined, for instance, by mutations of *CDKL5* or *FOXG1* genes, which are involved with the same biological pathways targeted by *MECP2* mutations.[12,25]

These various disorders have common and distinctive features regarding both the biological etiopathogenesis and clinical manifestations. Key etiopathogenic mechanisms include altered expression of the chloride potassium symporter 5 (KCC2), the vesicular glutamate transporter 1 (vGlut1), an orphan glutamate receptor subunit-1 (GluD1), the postsynaptic density protein 95 (PSD-95), and putatively the protein kinase B (Akt).[26] It is not yet clear whether Akt dysregulation is etiopathogenic for RTT or an associated feature, but reduced Akt signaling is reported consistently in RTT models and patients.[15] Moreover, several studies demonstrated beneficial effects of Akt activation obtained through increased signaling at various steps in the pathway CDKL5 – brain derived neurotrophic factor (BDNF) – FOXG1 that eventually regulates synaptic structure.[15] The pathway of GH – IGF-1 is also likely involved, due to its pleiotropic activity on the growth and trophism of all body tissues[27] and it has been extensively targeted by pharmacological trials, together with the NMDA glutamate receptors that mediate neuronal survival and health, especially in the context of energetic and functional stress.[28]

Therapeutic Approaches to RTT

The syndromic nature, the multi-systemic symptoms and the peculiar natural history make RTT difficult to treat, with limited therapeutic options available. However, despite its rarity, RTT represents one of the rare diseases that has the most advanced development of pharmacological treatments. Pharmacological studies on RTT dramatically increased over the past few decades, with more than 60 clinical trials finished or in progress. Yet, no treatment is available to cure the

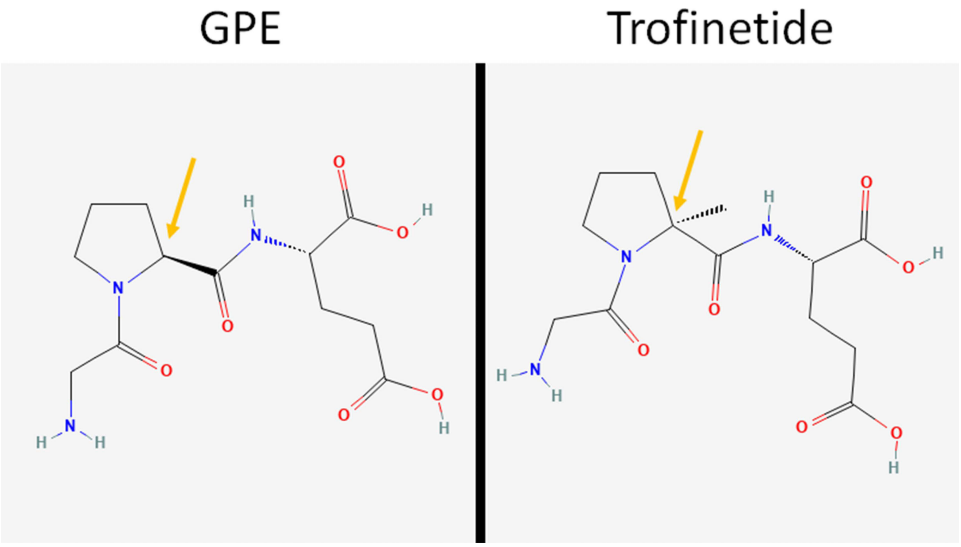

Figure 1 Chemical structures of GPE and Trofinetide.
Note: The orange arrow indicates the differences between trofinetide and GPE.

disease. Considering the mechanisms of action of the *MECP2* gene and the broad phenotypic spectrum, pharmacological strategies focused on targets downstream *MECP2* will likely require multiple drugs to effectively treat the full spectrum of RTT symptoms.[29] However, even the improvement of one of the core symptoms of the disease can be considered a valid objective and a useful pharmacological target. In this regard, we can include as main objectives of drugs the dysregulation of breathing, cardiac dysfunction, bone mass loss and fractures, sleep disorders, epilepsy and behavioral alterations. The novel and potentially advantageous approach chosen by the developers of trofinetide and other competing treatments is to try and compensate or revert the degenerative course of RTT, choosing to address directly the pathway of IGF-1 and on NMDA receptors, rather than focusing on single organ defects (Figure 1).[30]

Focus on IGF-1 and GPE

Considering the dysregulated genes and pathways mentioned above, growth hormone (GH), insulin-like growth factor 1 (IGF-1), and NMDA glutamate receptor targeting treatments were shown to produce benefits on survival and functional parameters, both in vitro and in vivo,[31,32] and ultimately in patients affected by RTT.[33]

IGF-1 and Neuronal Health

IGF-1 is a growth factor belonging to the GH/somatostatin axis. IGF-1 is produced mainly by energy-managing cells, including hepatocytes and pancreatic cells. The main actions of IGF-1 are exerted through activation of tyrosine kinase receptors in most peripheral tissues, and effects are related to the stimulation of growth and the inter-regulation of growth with energy metabolism. IGF-1 can cross the blood–brain barrier and have profound effects on neuronal growth and trophism, as well. IGF-1 is a driver of, and a factor associated with, neuronal regeneration,[34] survival and function in the adult brain.[35–38] It is recognized that IGF-1 concentrations decline with both ageing and degenerative brain disorders, acquired and congenital.[39,40]

The causative or consequent nature of IGF-1 variations is debated since it was found that specific brain regions connected with stimulus processing and memory (ie: hippocampus, cerebellum, subventricular zone, olfactory bulb) can produce autocrine/paracrine IGF-1 under stimulation by the growth hormone,[41–43] an effect directly correlated with regional potentiation of brain structure and function.

On a pharmacological level, the bulk of circulating IGF-1 is produced by constant hepatic synthesis, amounting to about 10–15 mg daily, which attains a stable plasma concentration around 10–30 nM. IGF-1 naturally has a quite short half-life of 8–16 hours, thus requiring constant release into the blood flow in order to sustain its pharmacological activity. Local brain

production of IGF-1 comes mainly from astrocytes and it appears to be affected by the same compensation and exhaustion dynamics of insulin.[9] Pathologies like Alzheimers' disease seem to be correlated with an initial peak of locally produced IGF-1 that putatively aims to maintain homeostasis in front of IGF-1 receptor hypo-response, eventually leading to functional exhaustion and hypo-production of IGF-1.[44]

Given this mechanism, IGF-1 replacement therapies in various neurodegenerative disorders work like insulin replacement in diabetes, and RTT can be seen as a type of neurodegenerative disorder characterized by deficits on several axes, inside which IGF-1 may provide an alternative source for activation of impaired signaling pathways. Indeed, IGF-1 has shown beneficial effects when given to patients with RTT.[9]

Pathways that are deficient in RTT and can be targeted by IGF-1 may include for instance: cholesterol metabolism since IGF-1 levels are correlated with HDL cholesterol levels in healthy adults,[45] and IGF-1 administration can ameliorate cholesterol metabolism in rodents;[46] oxidative stress, since in cultured cells, IGF-1 administration can prevent the buildup of reactive oxygen species by preserving mitochondrial function;[47] survival and trophism pathways that are interested also by effects from BDNF;[48] IGF-1 can increase mTOR mediated protein translation initiation in cells.[49]

A pilot open-label trial evaluating mecasermin (recombinant human IGF-1 approved for GH deficiency) for RTT patients found that it reduced apneas and behavioral symptoms.[33] A following double-blind placebo-controlled crossover study demonstrated that mecasermin is safe and improves repetitive behaviors and social communication; otherwise, mecasermin treatment did not produce significant improvements.[50] Moreover, electroencephalographic and respiratory parameters worsened and adverse events were reported in 80% of patients, half of whom required hospitalization due to severe adverse events.[50] This unpromising result may hamper further research on mecasermin for RTT.

GPE: A NMDA Receptor Modulator

Glycine-Proline-Glutamate (GPE) is one of the two degradation products of IGF-1, alongside des(1–3)IGF-1 that are produced by enzymatic cleavage.[1] With respect to previous research, it is likely that IGF-1 actions are mediated both by the whole IGF-1 and by its products des(1-3)-IGF-1 and GPE.[51]

While initial studies attributed the biological activity to the larger portion of IGF-1 it later became clear that also the smaller GPE fragment retained noticeable and distinct effects.[52] While des(1–3)IGF-1 can bind IGF-1 receptors similarly to the parent hormone, the activity proper of GPE was demonstrated to be independent of IGF-1 receptors.[53] It was demonstrated to be mediated by NMDA receptors, which is unsurprising, given that GPE extremities are the known ligands of NMDA receptors glycine and glutamate. Indeed, when competing in vitro with the NMDA agonist AP5, only IGF-1 and GPE, but not des(1–3)IGF-1 displayed ligand activity.[54] Moreover, the non-competitive NMDA receptor antagonist MK801 was shown to inhibit the effect of GPE on glial proliferation in vitro,[55] and in animal models of NMDA-induced injury, the anti-toxic effect of GPE was similar to that of MK801,[56] overall indicating that NMDA, MK801 and GPE all bind the same receptor. Indeed, GPE also has effects on brain regions that do not respond to IGF-1, like the CA1–2 sub-regions of the hippocampus, rich in NMDA receptors.[57,58]

A role for NMDA receptors in causing RTT symptoms is supported also by the results of a placebo-controlled trial on dextromethorphan for RTT. Dextromethorphan that apparently blocks NMDA receptors caused dose-dependent improvements in seizures, receptive language, and behavioral hyperactivity, but no significant improvement in global clinical severity.[59]

The neuroprotective effects of GPE against ischemia-reperfusion neuronal injury are mediated by a series of mechanisms including inhibition of apoptotic and non-apoptotic death pathways;[60] blood flow increase via NOS induction;[58] better tolerance to glutamate toxicity and increased glutamate clearance by glutamic acid decarboxylase;[58] and maintaining astrocyte integrity.[60] Astrocytes may be key mediators of GPE effects, as human astrocytes bearing RTT mutations can induce phenotypes typical of RTT in co-cultured wild-type neurons, and both IGF-1 and GPE are effective in rescuing some of the caused phenotypes.[61]

Pharmacokinetic considerations on GPE as compared with the whole IGF-1 mainly regard distribution and elimination. It is important to note that most IGF-1 receptors and binding proteins are outside the central nervous system and are involved with metabolic regulations and tissue growth. These peripheral IGF-1 receptors and binding proteins would act as buffers sequestering administered IGF-1, thus lowering its dose-effectiveness inside the central nervous system.

Instead, peripheral tissues lack NMDA receptors, therefore, GPE administration is subject to negligible off-target binding outside the central nervous system. Moreover, the smaller molecular weight of GPE, as compared with IGF-1, would suggest a better distribution to the central nervous system. However, as compared with IGF-1, GPE has lower stability, which is counted in minutes of half-life in the plasma and around half an hour in the brain and cerebrospinal fluid.[62,63]

Although repeated infusion of GPE is required to reach significant concentrations in the brain of rodents, daily intraperitoneal injections of GPE were shown to rescue the characteristic phenotypes of the RTT mouse model, including reduced brain weight, reduced PSD95 expression and spine density in the motor cortex, reduced excitatory postsynaptic currents, and synaptic immaturity.[64] In an effort to find a stable and efficacious analog of GPE, molecules like G-2mPE[65] and cyclic-Gly-Pro[66] and NNZ-2591[67] were developed and tested in vitro and in vivo, all showing different issues and advantages and not reaching a PK-PD profile suitable for a potential human use.[68,69]

Trofinetide: A Newly Approved GPE-Derivative with Longer Half-Life

Trofinetide Development and Approval Status

Trofinetide, initially known as NNZ-2566, is a GPE derivative modified with the aim to extend its half-life (Figure 2).

Trofinetide has been extensively studied in vivo for neuroprotection following penetrating brain injury and stroke[70–74] and its mechanism of action was identified in reducing neuroinflammation in these animal models.[73,74]

Trofinetide was then tested in murine models of Fragile X Syndrome, finding a positive effect on behavioral and neuropsychological features, but also on synaptic architecture and signal transduction pathways.[75] This was in line with the effects previously reported for GPE and on the same pathways that are impaired in the context of RTT and related syndromes.

After showing promising efficacy in vivo for Fragile X Syndrome, trofinetide was tested in healthy volunteers to assess its safety and pharmacokinetics as given by oral and injectable administrations, which led to finding a plasma half-life of up to 1.4 hours,[76] which is considerably longer than that of GPE. Trofinetide was then developed for human use with a tentative indication in RTT, until reaching marketing authorization as a first-in-class medication.

The safety, tolerability and potential efficacy of oral trofinetide in RTT were evaluated in two Phase 2 double-blind, placebo-controlled, dose-escalation trials in adolescents and adults (NCT01703533)[77] and then in children and adolescents (NCT02715115).[23]

On 10th March 2023, the Food and Drug Administration (FDA) approved oral trofinetide in the US for the treatment of RTT in adult and pediatric patients aged 2 years and older, based on data from two clinical trials performed by Acadia Pharmaceuticals: LAVENDER (NCT04181723)[78] and DAFFODIL (NCT02715115).[79] The US patent for treating RTT with trofinetide has an expiration date in 2032.[1] Alongside granting approval, the FDA considered that the safety database was adequate and the risks associated with trofinetide treatment are acceptable for the indicated population.

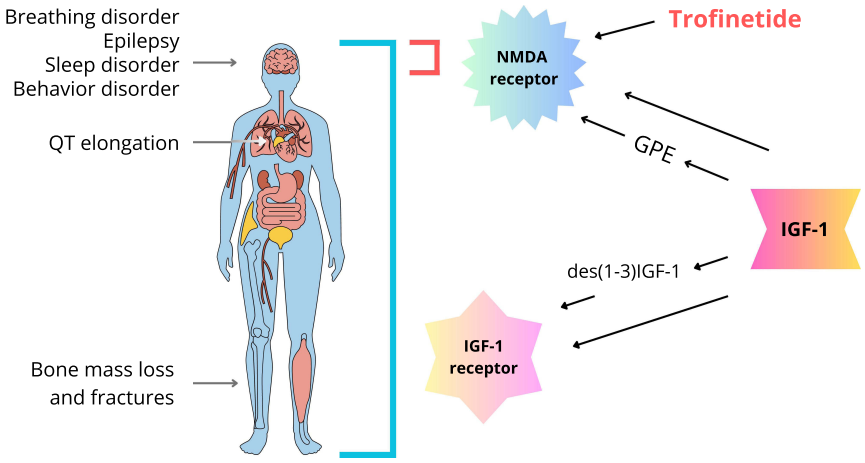

Figure 2 Summary of symptoms and Trofinetide action in Rett Syndrome.

However, the FDA determined that an analysis of spontaneously reported post-marketing adverse events would not be sufficient to prove or disprove neither a serious and unexpected risk of carcinogenicity following exposure to trofinetide nor a serious and unexpected risk of drug–drug interaction. Therefore, the FDA has requested Acadia Pharmaceuticals to conduct two carcinogenicity studies in mice and rats, an in vitro drug interaction study focused on CYP2B6, an in vivo drug interaction study on OATP1B1 and OATP1B3 and a pharmacokinetics clinical trial to evaluate the effect of moderate renal impairment on the exposure to trofinetide.[1]

Clinical Studies and Core Efficacy Endpoints

A recent meta-analysis[14] included the three preliminary RCTs conducted by Acadia Pharmaceuticals on trofinetide in RTT patients. Glaze 2017[77] examined adolescents and adults (15–25 years old) and used two fixed doses (35, 70 mg/Kg), measuring efficacy outcomes at day 54. Glaze 2019[23] examined children and adolescents (5–15 years old) and used three fixed doses (50, 100, 200 mg/Kg) measuring efficacy outcomes at day 66. Neul 2023[78] examined patients between the ages of 5 and 20 and used a dose ranging between 200 and 500 mg/Kg per day, measuring efficacy outcomes at 12 weeks. Meta-analytical results indicate that trofinetide improved CGI-I scores and RSBQ scores; particularly improved subscores included impaired communication, compulsive behaviors, and emotional disorders, which improved the lives of patients and their families.[14]

The primary evidence of effectiveness for application of trofinetide in RTT is based on the analysis of clinical efficacy endpoints from the 3 month, multicenter, double-blind, placebo-controlled Phase 3 LAVENDER trial from Acadia Pharmaceuticals.[78] A total of 187 subjects were randomized 1:1 to receive either trofinetide in a weight-based dosing (N = 93) or placebo (N = 94) twice daily. Subjects were required to be between 5 and 20 years of age and have a genetically confirmed diagnosis of RTT. The first co-primary efficacy endpoint was the change from baseline to week 12 on the Rett Syndrome Behavior Questionnaire (RSBQ) total score, as compared to placebo. Regarding this endpoint, the FDA recommended Acadia Pharmaceuticals to include a clinical co-primary endpoint to support their results, because RSBQ scores can be significantly different even when the changes are not clinically meaningful. For this reason, the Clinician's Global Impression of Improvement (CGI-I) was included as a co-primary endpoint. CGI-I is a clinician-completed assessment of how much the individual's illness has improved/worsened relative to a baseline state, scored using a standardized 7-point grid. LAVENDER also evaluated the Communication and Symbolic Behavior Scales Developmental Profile-infant-toddler social composite score (CSBS-DP-IT-SCS) as a secondary endpoint. It is a screening assessment to detect potential communication defects in children, which consists of 24 questions divided into 7 subscales referring to the communicative sphere: emotion and eye gaze, communication, gestures, sounds, words, understanding and object use. After treatment, there was a significant difference between trofinetide-treated and placebo-treated patients favoring trofinetide, on both the co-primary endpoints CGI-I ($p = 0.003$) and RSBQ ($p = 0.018$). For the latter, significant effects were observed on the General Mood subscale ($p = 0.007$) and the Repetitive Face Movement subscale ($p = 0.047$). Nominal improvements were also observed in other areas, such as Breathing problems, Walking/Standing, Nighttime behaviors and Seizures, but these were not significant. Trofinetide appears to have no effect on the other domains evaluated by the RSBQ.[23]

These results were also supported by a significant improvement in the secondary endpoint CSBS-DP-IT-SCS ($p = 0.006$).[78]

Acadia Pharmaceuticals also conducted an open-label study (DAFFODIL) to evaluate pharmacokinetics and safety of trofinetide in RTT patients between 2 and 4 years of age. The mean CGI-I score at week 12 corresponded to "much improved" with respect to the baseline. The interim PK analysis based on data from 13 children treated with trofinetide for 12 weeks demonstrated a PK exposure to trofinetide and safety profiles similar to those of pediatric patients ≥5 years of age and of adults, leading to the approval of trofinetide for age 2 and above.[79]

Acadia Pharmaceuticals then conducted two open-label extension trials on patients who concluded LAVENDER, named LILAC and LILAC-2. LILAC demonstrated continued benefits on both RSBQ and CGI-I from open-label treatment with trofinetide, for up to 40 additional weeks after the end of LAVENDER[11] and LILAC-2 for other 32 additional weeks after the end of LILAC.[11] Overall, treatment for 84 weeks with trofinetide demonstrated continued

improvement on both co-primary endpoints, with the same adverse effects of short-term treatment. However, it is worth noting that, at 84 weeks, 59.4% CGI-I and 88% RSBQ evaluations had missing values.

Adverse Events

The risks associated with trofinetide use did not preclude approval. The most common adverse events were of gastrointestinal nature, including serious diarrhea and vomiting. The FDA recommended that warnings for the clinical significance of diarrhea and weight loss were to be added to the product label.

Based on the three meta-analyzed preliminary studies,[14] diarrhea ($p = 0.06$) and vomiting ($p = 0.002$) were the only adverse events associated with trofinetide treatment. Considering the LAVENDER trial, the most common adverse reactions reported in at least 5% of trofinetide-treated subjects were diarrhea (82%), vomiting (29%), fever (9%), seizure (9%), anxiety (8%), decreased appetite (8%), fatigue (8%), and nasopharyngitis (5%). Diarrhea occurred in nearly 85% of subjects on long-term treatment with trofinetide, while 12% of subjects experienced a loss of greater than 7% of body weight. This is a clinically meaningful risk in a pediatric population.[78]

Other Therapeutic Strategies: Restoration of MECP2 Expression

These approaches include gene therapy and reactivation of the inactive X chromosome. They have an unclear window of opportunity before which they have to be carried out, in order to produce a significant correction in the degenerative pathway typical of RTT progression. Restoring *MECP2* expression in late life may improve some functional phenotypes but may not improve most structural changes to the brain and/or organ architecture that have already occurred. Gene therapies for RTT are reviewed elsewhere.[4]

Other Therapeutic Strategies: Restoration of Molecular Pathways Downstream MECP2-Induced Genes in Peripheral Tissues

There is no ascertained common mechanism of action underlying the phenotypes described below that target heart and bone. However, it is likely that a pleiotropic effect of loss of signaling downstream the GH receptor is involved.

Cardiac Dysfunction

The main cardiac dysfunction present in people with RTT is the prolonged QT interval, ie the timing between ventricle depolarization and repolarization is delayed in RTT patients as compared to age-matched healthy people. The state-of-the-art treatments for QT prolongation in the general population are beta-blockers, but they were not effective in preventing cardiac alterations in *MECP2* null mice.[4] This suggests that standard treatment is insufficient in the context of cardiac dysfunctions descending from RTT. In view of the lack of efficacy of available treatments, current recommendations are to avoid QT-elongating drugs in people with RTT that have developed prolonged QT intervals, in order to help avoid cardiac dysfunction.[5]

Bone Mineral Loss and Fractures

Bone mineral loss and increased likelihood of fractures is an early concern in the RTT population, as opposed to typically developing women, who encounter increased risks of fractures only after menopause. Prophylactic treatment with vitamin D needs to be considered in people with RTT.[20] Another treatment proposed to RTT patients with mineral loss is bisphosphonates, which inhibit bone resorption and therefore prevent the reduction of mineralization in bone.[20] However, in clinical practice bisphosphonates are rarely used due to their adverse effects, such as abdominal pain, inducing osteonecrosis and increasing the risk of non-vertebral fractures with long-term use. This characteristic must be considered in the evaluation of drug–disease interactions considering, for example, that some antiepileptic drugs alter bone metabolism, which increases the risk of fractures. Carbamazepine, phenytoin, and valproic acid also reduce blood calcium levels, further increasing the risk of fractures in RTT patients with epilepsy.[80,81]

Other Therapeutic Strategies: Restoration of Molecular Pathways Downstream MECP2-Induced Genes in the Brain

Drugs targeting molecular pathways downstream genes induced by *MECP2* aim to restore mainly the excitatory–inhibitory synaptic balance in specific neural circuits. Considered pathways and pharmacological targets include neurotransmitter and neuromodulator systems such as the noradrenergic, serotonergic, glutamatergic, GABAergic, and cholinergic signaling; GH, BDNF and IGF-1 signaling; energy metabolism including the cholesterol biosynthesis pathway; mitochondrial function and oxidative stress; Akt/mTOR mediated regulation of protein translation initiation.[6–8,15,29,82] Recently, a role of the gut microbiota was suggested in the progression and symptoms of RTT, providing an additional pharmacological target.[10]

Overall, the pathways involving GH, IGF-1 and NMDA receptors have been implicated with altered neuronal functions in RTT and related syndromes.

Breathing

Dysregulation of breathing is a core feature of RTT in up to 93% of patients, it significantly impacts quality of life, and it is thought to contribute to early mortality in some patients.[83] Common issues are hyperventilation, apnea, breath holding, and air swallowing that occurs when waking up. At the origin of breathing dysregulation, there are abnormalities in brain stem connections, with impaired development of the neuronal circuitry responsible for setting the respiratory rhythm, which is secondary to deletion of *MECP2* in GABAergic interneurons[83] and was shown to respond to BDNF administration in *MECP2* mice.[13,84] Several molecules appeared as promising in preclinical and clinical studies to reduce breathing abnormalities and sarizotan (a 5-HT1a agonist and a dopamine D2–like agonist/partial agonist) reduced apneas in mouse models of RTT, but a study on efficacy, safety, and tolerability of sarizotan in RTT with respiratory symptoms was terminated early during the double-blind period because it did not demonstrate evidence of efficacy on the reduction of respiratory abnormalities, nor on the caregiver-rated impression of change.[13,17,84,85] The noradrenaline uptake inhibitor desipramine[86] was also tested, showing no indication of efficacy.[87] A study to assess the safety and efficacy of fingolimod, which should increase levels of brain-derived neurotrophic factor (BDNF), also had negative results because changes in BDNF levels in the CSF and serum were not found and clinical outcomes were not met.[88]

Epilepsy

Epilepsy regards approximately 60–80% of RTT individuals, with heterogeneous semiology (focal or generalized), and it appears to be an age-related event, as patients 6 to 12 years of age tend to be at highest risk for seizure development; it is debilitating and often drug-resistant.[89] *MECP2* mutant mice have an imbalance of excitation-to-inhibition in brain regions known to contribute to epilepsy and seizure disorders.[89–91] Epilepsy is considered a major issue in RTT and is managed like in other rare diseases and developmental and epileptic encephalopathies.[92] Tolerability is the main issue with anti-seizure medications, which are associated with many different adverse drug effects. For instance, the use of lamotrigine may cause serious rash or levetiracetam may cause behavioral disturbance;[93–95] therefore, limiting their use to RTT patients with an active seizure disorder would minimize the number of patients with adverse effects, although the neurological best practice would be to prevent seizures with prophylactic treatment.[94,95] Lastly, since RTT patients may be taking many medications, determining drug–drug interactions upfront is also mandatory.[81] For instance, carbamazepine is a known CYP450 inducer and may cause a drug–drug interaction by increasing the clearance and decreasing the blood concentration of other drugs that the patient may be taking.[81,96] Secondarily, efficacy is of concern in RTT patients with epilepsy. Sodium valproate has the highest number of positive recommendations for different kinds of epilepsy.[81,94–96] However, no recommendations of a specific treatment were made for RTT -associated epilepsy. As in the general population, 30% of patients with RTT also have drug-resistant epilepsy.[81] In these cases, ketogenic diet or vagus nerve stimulation are considered, alongside innovative drug therapies below.[96,97]

Glatiramer acetate, which is labeled to treat multiple sclerosis by acting on immune cell activation, leads to delayed onset of RTT symptoms and increased BDNF levels.[98] A phase 2 open-label trial to test glatiramer in patients with RTT demonstrated that epileptiform discharges were decreased in four patients, but the trial was terminated due to the development of life-threatening post-injection responses in 3 out of 14 patients.[98,99]

Cannabidiol is an inhibitor of fatty acid aryl hydroxylase, which leads to increased concentrations of endogenous cannabinoids, and also a GPR55 antagonist, overall dampening neuronal excitability.[100] Cannabidiol is efficacious for various syndromic drug-resistant epilepsies, such as Lennox–Gastaut and Dravet, Aicardi, Dup15q, and Doose syndromes, SYNGAP1 encephalopathy, epilepsy with myoclonic absences, and atypical RTT as CDKL5-related encephalopathy, with some reported benefits.[100–102] A long-term safety study of cannabidiol oral solution showed seizure frequency was reduced. However, populations suffered from serious adverse side effects such as diarrhea, vomiting, fatigue, pyrexia and somnolence. The study was terminated early due to the COVID-19 pandemic and recruitment challenges.[103] The available data on cannabinoids used for RTT patients show possible promising effects of cannabidivarin (CBDV), the propyl analog of cannabidiol (CBD). CBDV and CBV are safe and well tolerated.[17,104] They reduce the incidence of seizures, agitation and/or anxiety attacks.[100] These drugs do not alter the safety and effectiveness of diazepam or clobazam, which supports concomitant use in selected patients.[104–108]

Ganaxolone is a synthetic analog of the neuroactive steroid allopregnanolone that acts as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors, thus having antiseizure properties in preclinical and clinical studies.[109] Ganaxolone is now approved for epilepsy associated with the RTT-like CDKL5 syndrome, in patients aged two years and older, which is of immediate interest also for patients with RTT, although there is yet no specific indication of use.[110,111] Ganaxolone might also improve other aspects of children's wellbeing, secondary to a better control of seizures.

Sleep Disorders

An open-label trial of L-carnitine, an amino acid derivative of methionine and lysine that is required for energy metabolism, showed improved sleep maintenance in a subset of RTT patients.[112]

Additional therapeutic alternatives, not specifically tested for RTT, include the serotonin receptor modulator trazodone,[81] the sedating agent chloral hydrate,[113,114] and melatonin,[115] whereas benzodiazepines are not recommended in RTT, given the respiratory issues of some of these patients.[81,116]

Behavior Disorders

Disorders of behavior in RTT patients include anxiety, fearful behaviors, various mood disturbances, aggression and self-harm.[117] The scientific literature is poor on this topic, and treatment usually follows what is indicated for disorders associated with autism spectrum disorders and other genetic syndromes, even if RTT presents with peculiar and distinct characteristics, especially with regard to the risk of adverse effects.[118,119] The most successful management of anxiety and mood dysregulations is with selective serotonin modulators, like mirtazapine that appear to protect against disease progression and improve motor, sensory and behavioral symptoms.[120] In support of this clinical evidence, it was demonstrated that serotonin levels are reduced in the hippocampus of *MECP2* null mice, as compared to those of wild-type mice.[5,120] Aggression and self-harm, irritability and impairments in socialization are anecdotally treated using risperidone and other antipsychotic sedating drugs, at the cost of several adverse metabolic and cardiac effects.[120] Moreover, the sigma-1 receptor antagonist blarcamesine appeared to improve RSBQ and CGI-I in a 7-week double-blind, randomized, placebo-controlled clinical trial (NCT03758924) sponsored by Anavex Life Science Corp. In this study, low-dose blarcamesine produced a clinically significant effect in reducing symptoms of RTT ($p < 0.007$) and led to a significant slowing of disease progression ($p < 0.007$).[121]

Shortcomings of Other Drug Therapies

With the notable exception of gene therapy (yet in early clinical research), the pharmacological approaches described above and summarized (Table 2) are mostly symptomatic, they are used in clinical practice to try and improve the quality of life of families and patients, but they have no impact on the degenerative natural history of the disease. The result is

Table 2 Treatments for RTT Under Clinical Development with Published Results

Authors (year)	Active Substance	Study Design	RTT Subjects	Results
Djukic A et al (2016)[4]	Glatiramer Acetate	Prospective open label trial – Phase II	10	Gait velocity, memory, and the breath holding index improved significantly. Epileptiform discharges decreased. There was a trend towards improved quality of life, which did not reach statistical significance.
Nissenkorn A et al (2016)[5]	Glatiramer Acetate	Open label trial – phase II	14	4 patients developed an exaggerated immediate postinjection response, which was experienced as life threatening in 3 patients, necessitating arrest of the trial.
Desnous B et al (2023)[6]	Cannabidiol - Clobazam	Longitudinal observational study	10	CBD is well tolerated and may increase the efficacy of clobazam alone. It reduced the incidence of seizures, agitation and/or anxiety attacks. In addition, improvement in spasticity was reported in 4 patients.
Peters JM et al (2023)[7]	Cannabidiol - Diazepam	Long-term safety study – Phase III	21 out of 163	CBD does not alter the safety and effectiveness of diazepam nasal spray and supports concomitant use in appropriate patients.
Tarquinio D et al (2023)[8]	Diazepam	Long-term safety study – phase III	16 out of 64	In patients with developmental epileptic encephalopathies, diazepam nasal spray demonstrated a consistent safety profile.
Smith-Hicks et al (2017)[9]	Dextro-methorphan	Prospective randomized open-label trial	38	Dextromethorphan is safe. Statistically significant dose-dependent improvements were seen in clinical seizures, receptive language, and behavioral hyperactivity. There was no significant improvement in global clinical severity.
Flores Gutiérrez J et al (2020)[10]	Mirtazapine	Retrospective study	80	Mirtazapine is well tolerated and it protects from disease progression and improves motor, sensory, and behavioral symptoms.
Hurley EN et al (2022)[11]	Cannabidivarin	Clinical trial – phase I	5	CBDV is safe and well tolerated. The main result was a reduction in seizure frequency. No significant changes were observed in the EEG or in symptoms unrelated to RTT.
Khwaja OS et al (2014)[12]	Mecasermin	Unblinded study - phase I	9 out of 12	Mecasermin is safe and well tolerated in girls with RTT and, as demonstrated in preclinical studies, improves certain breathing and behavioral abnormalities.
Ette et al (2023)[13]	Blarcamesine	Double-blind, randomized study – phase II	25	Low-dose blarcamesine produced a clinically significant effect in reducing symptoms of RTT and it led to a significant slowdown in disease progression.
Mancini et al (2017)[14]	Desipramine	Double-blind, randomized study – phase II	34	Not significant difference between the effects of placebo on the apnea-hypopnea index at 6 months. Neither did we show significant effects for other respiratory parameters.
Ellaway et al (2021)[15]	L-carnitine	Open label trial	21	There was a significant improvement in outcome for the cases compared with the controls in sleep efficiency, energy level, communication skills and expressive speech.

Table 3 Other Treatments for RTT Under Clinical Development Without Published Results

Drug	Intended Target	Mechanism	Phase
AMO-04 Tianeptine	Glutamate receptors	Modulator	2
Ketamine	GRIN receptors	Modulator	2
VYNT-0126	Not disclosed	Not disclosed	2
NGN-401	*MECP2* Gene on inactive X chromosome	EXACT transgene regulation technology	1–2
TSHA-102	*MECP2* Gene	scAAV9 vector carrying recombinant *MECP2*	1–2
ACP-2591	IGF-1 receptors	Modulator	1
F-15599 NLX-101	5-HT1A cortical receptors	Highly selective agonist	1
Fingolimod	Sphingosine 1-phosphate receptor 1	Modulator	1
Vatiquinone	15-Lipoxygenase	Selective inhibitor	–
GXV001	Not disclosed	Not disclosed	–
Donepezil	AChE	Inhibitor	T
Lovastatin	HMGCR	Inhibitor	T
Sarizotan	5-HT1a and DRD2	Agonist/partial agonist	T

Note: – : not clear, T : terminated.

a mostly off-label and heterogeneous use of pharmacological mono- and poly-therapy, which leads to high rates of failure and a severely increased risk of adverse effects and drug–drug and drug–disease interactions. Other treatments are undergoing development, but clinical results are not yet available (Table 3).

Discussion

Future Perspectives for Trofinetide

The currently published clinical studies on trofinetide show limitations that could be overcome through future research and with some adjustments: long-term follow-up studies should investigate the real-life impact and sustainability of trofinetide treatment, especially regarding its formulation and dosing, which leads to gastrointestinal adverse events. The duration and economic impact of treatment with trofinetide should also be investigated, possibly with HTA assessments. It would also be necessary to identify the optimal and most tolerable dosing of trofinetide for different types of RTT and related syndromes, as well as different RTT stages and presentations. This is mandatory in order to tailor drug administration to each patient and predict drug response, an important issue with rare diseases. Biomarkers should be determined to pinpoint what physiological, neurocognitive or behavioral mechanisms trofinetide tackles. These aspects would allow for a more patient-specific treatment.

Current Place in Therapy of Trofinetide

Trofinetide, a first-in-class medication approved by the FDA for the treatment of RTT in people aged 2 years and above, is demonstrated to be relatively tolerable, except for moderate/severe gastrointestinal symptoms. Trofinetide is effective on some, but not all, symptoms of RTT, as shown by the significant improvement only on some sub-scales of the RSBQ, as discussed above (reviewed in a recent meta-analysis).[122] This selectivity of effect likely depends on the absence of binding to IGF-1 receptors. Regarding the methods of administration and management of the drug on the practical side, trofinetide is supplied as 200 mg/mL oral solution in a 450 mL bottle, costing around ten thousand USD and containing enough medication for ten, down to three days, depending on patients' weight. These aspects may constitute a barrier to use, given the chronic nature of RTT. In clinical practice, trofinetide may be suitable as a stand-alone or combined

treatment, depending on each patient's needs. In order to manage these further issues, additional pharmacokinetic and drug–drug interaction studies are under way, as requested by the FDA, to ensure it can be used also in combination with other treatments in complex clinical settings.

It is yet unclear whether trofinetide may be a symptomatic or curative treatment. In theory, these may both be possible, depending on the age of treated patients: ie trofinetide may reduce the progression of RTT for patients below a certain age threshold, acting through the correction of NMDA-mediated neuronal network structuring; trofinetide may only ameliorate RTT symptoms above a certain threshold age, allowing neuronal networks to function better.

Further phase 3 or 4 efficacy studies will also be needed to re-determine the place in therapy of trofinetide when competitors will enter the market and also with respect to other IGF-1 or NMDA targeting therapies. For instance, mecasermin, the recombinant human IGF-1 analog, may be further investigated as a potential alternative to trofinetide given its non-novel status and possibly much lower costs; in this perspective, however, the preliminary unsatisfying results obtained by mecasermin would require scrutiny and proper re-assessment in comparative clinicaltrials.in view of the significant effect of trofinetide. Overall, trofinetide is a crucial new tool for the pharmacological treatment of RTT and related conditions. Its use is likely to be considerably improved during the first years of marketing.

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