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DAYBUE® (trofinetide): Questionnaires Used in the LOTUS Study

This letter is provided in response to your specific request for information regarding the questionnaires used in the LOTUS Phase 4 study of trofinetide in individuals with Rett syndrome.

Summary

- In the ongoing Phase 4 LOTUS study enrolling individuals prescribed trofinetide under routine clinical care, the effectiveness and tolerability of trofinetide treatment are primarily being assessed using three electronic caregiver-reported measures: the **Behavioral Improvement Questionnaire**, **Quality-of-Life Inventory–Disability (QI–Disability) scale**, and the **Gastrointestinal (GI) Health Questionnaire**.[1]
- Caregiver responses to the **Rett Syndrome Behaviour Questionnaire (RSBQ)** are being collected in a subgroup of approximately 30 patients as part of a video substudy that was added to the LOTUS protocol in 2025.[2]

LOTUS (ACP-2566-014)

This is an ongoing Phase 4, prospective, observational, real-world study involving caregivers of adults or pediatric patients of either sex who are prescribed trofinetide under routine clinical care in the United States (US). The effectiveness and tolerability of trofinetide treatment are primarily being assessed using three electronic caregiver-reported measures,[1] which are available in English and US Spanish (**Table 1**). Two of the three questionnaires (Behavioral Improvement and GI Health) were created specifically for this study and have not been validated in individuals with Rett syndrome.[1] Caregivers can download their responses to these measures as PDFs to share with their Healthcare Providers.

Table 1. Electronic Caregiver-reported Measures[3]

Electronic caregiver-reported measure	Description	Frequency
Behavioral Improvement Questionnaire	Selection of perceived behavioral improvements since starting trofinetide, across multiple domains	Collected monthly for 6 months, and every 3 months thereafter
QI–Disability scale[4]	A measure of quality-of-life for children and adolescents with intellectual disability	Collected monthly for 6 months, and every 3 months thereafter
GI Health Questionnaire	Information on GI symptoms occurrence, frequency, and management	Collected weekly for 12 weeks, then monthly for 3 months, then every 3 months

Abbreviations: GI=gastrointestinal; QI–Disability=Quality-of-Life Inventory–Disability.

A video substudy was added to the LOTUS protocol in 2025. This substudy in approximately 30 trofinetide-naive patients is being conducted to assess function and behavior through video- and audio-based evaluation and to collect caregiver responses to the RSBQ. Patients in the video substudy will be followed from the time of the baseline video assessment to Month 18 post-initiation of trofinetide.[2]

Behavioral Improvement Questionnaire

This questionnaire is a new measure in which caregivers report 'yes' or 'no' to "Have you observed improvements that are new and/or maintained in your loved one's Rett syndrome symptoms as compared to before starting trofinetide?" A 'yes' answer enabled identification of areas of improvement from the checklist (**Table 2**).[1] The checklist of items was generated by compiling potential improvements from the items in the RSBQ, the top caregiver concerns from the US Natural History Study, and the RTT community list of symptoms in the Voice of the Patient Report.[5-7]

Table 2. Behavioral Improvement Questionnaire Questions[3]

1. Have you observed improvements that are new and/or maintained in your loved one's Rett symptoms as compared to before starting DAYBUE?
2. Please check all areas in which improvements that are new and/or maintained have been observed:
   - ☐ Non-verbal communication: gestures, use of eye contact, etc.
   - ☐ Communication tools: use of AAC, choice cards, iPad, etc.
   - ☐ Verbal communication
   - ☐ Alertness
   - ☐ Social interaction/connectedness
   - ☐ Purposeful use of hands
   - ☐ Repetitive movements (e.g., wringing hands, mouthing hands)
   - ☐ Muscle tone abnormalities (high tone/rigidity)
   - ☐ Mobility or balance (e.g., walking, crawling, weight bearing for transfers)
   - ☐ Sleep
   - ☐ Breathing irregularities
   - ☐ Behavioral problems
   - ☐ Mood
   - ☐ Eating/swallowing
   - ☐ Grinding teeth
   - ☐ Other (please describe in free text field below)

     You selected Other above. Please describe. _____.

Abbreviation: AAC=Augmentative and Alternative Communication.

QI–Disability Scale

This scale was developed by Downs et al. as a measure of quality-of-life for school-aged children and adolescents with intellectual disability,[1] and has been validated through psychometric testing for adults with Rett syndrome.[4] It includes 32 questions across 6 domains: social interaction, physical health, independence, positive emotions, leisure and the outdoors, and negative emotions (**Table 3**),[1] and takes approximately 10 minutes to complete.[3]

Table 3. QI–Disability Questions[2]

Over the past month, how often has your child…
Social Interaction
1. Expressed happiness when they were understood
2. Appeared relaxed when making eye contact
3. Initiated greetings with people verbally or nonverbally (e.g., eye contact)
4. Enjoyed being included
5. Enjoyed the social experience of mealtimes
6. Responded positively when others paid attention to them (e.g., your child smiled, showed interest)
7. Showed pleasure or excitement when looking forward to activities (e.g., going to school, outings, events)
Feelings and Emotions
8. Been in a good mood
9. Smiled or brightened their facial expression
10. Showed happiness through body language (e.g., making eye contact, body facing others)
11. Showed cheeky or comical mannerisms (e.g., laughed, giggled)
12. Been unsettled without an apparent reason
13. Showed aggression (e.g., hitting, kicking, using offensive language, being destructive)
14. Appeared angry or upset (e.g., crying, screaming, moving, or stiffening the body)
15. Become withdrawn with a low mood
16. Deliberately hurt themselves
17. Expressed discomfort with changes in routine (e.g., carers, school, respite, out-of-home care)
18. Showed signs of being anxious or agitated (e.g., teeth grinding, fast breathing, avoidance)
Physical Health
19. Had enough energy to participate in daily routines and activities
20. Kept in good general health (e.g., avoided coughs, colds, fever)
21. Slept well during the night
22. Been alert and aware during the day
Activities and the Outdoors
23. Enjoyed moving their body (e.g., walking, swinging)
24. Enjoyed feeling steady or stable during physical activities (e.g., sitting, standing, bike riding)
25. Enjoyed physical activities (e.g., going out for a walk, swimming, dancing)
26. Enjoyed going on outing in the community (e.g., shopping, party, sports, theatre)
27. Enjoyed spending time outdoors (e.g., contact with water, grass, wind, sunshine)
Daily Life
28. Expressed their needs (e.g., hunger, thirst, toileting)
29. Made their own choices for activities or things they enjoy (e.g., DVDs, toys)
30. Helped to complete routine activities (e.g., dressing, feeding, chores around the house)
31. Enjoyed making things with their hands – can be with help (e.g., building blocks, painting, cooking)
32. Enjoyed using technology (e.g., computer, tablet, applications on phones)

Abbreviation: QI–Disability=Quality-of-Life Inventory–Disability.

Caregiver response options are never, rarely, sometimes, often, and very often. Each item is rated on a 5-point Likert scale, and all items are linearly transformed to a scale of 0 to 100. Specifically, the response "never" is given the value 0, "rarely" is given the value 25, sometime" is given the value 50, "often" is given the value 75, and the response "very often" is given the value 100. Domain scores are calculated by summing all item scores and dividing that value by the number of items. Items in the negative emotions domain are reverse coded. Higher scores on the QI–Disability represent better quality-of-life.[1,3] It has been estimated that a change of nearly 5 points on the QI–Disability would be meaningful.[8]

GI Health Questionnaire

This questionnaire is a new measure designed to assess GI health including dosing timing and amount, incidence of diarrhea and vomiting, the type of stool formation over the past 3 days, specifics about diarrhea frequency, and GI management strategies for preventing or managing diarrhea employed by caregivers.[1] The questionnaire was administered at enrollment (**Table 4**) and after initiation of trofinetide treatment (**Table 5**).[3]

Table 4. Baseline: GI Health Questionnaire Questions[3]

1. Has your loved one started taking DAYBUE yet?
2. Date of first DAYBUE dose
3. Rett syndrome type (Classic; Atypical; Does not meet the diagnostic criteria for either)
4. Rett syndrome diagnosis date
5. Last menstrual period: please indicate the first day of your loved one's last period (Select this answer to input an approximate date of last menstrual period; No regular period for the past month [for example, spotting]; N/A [does not menstruate])
6. Approximate date of last menstrual period
7. On average, how many diaper changes due to bowel movements were usually required **per day** over the past month? (0, 1, 2, 3, 4, 5, 6, 7, 8, >8)
8. On average, how many toilet trips due to bowel movements are usually required per day over the past month? (0, 1, 2, 3, 4, 5, 6, 7, 8, >8)
9. Have you needed to manage gastrointestinal issues (e.g., constipation, diarrhea, vomiting) over the past month? *Please check all that apply*:
   - ☐ Constipation
   - ☐ Vomiting
   - ☐ Diarrhea
   - ☐ Other

Abbreviation: GI=gastrointestinal.

Table 5. After initiation of DAYBUE: GI Health Questionnaire Questions[2]

Dosing

We would like to know how you are giving the medication to your loved one.

1. Please indicate how many times per day you give DAYBUE to your loved one (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10)

For today's doses, please select the approximate time and number of mL for each dose that you have given or plan to give your loved one today:

2. Time of 1st dose
3. Volume of 1st dose (mL) (0-5, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65, 66-70, 71-75, 76-80)

[The caregiver to enter time and volume for the number of doses identified in Question 1]

Diarrhea

4. Which strategies have you tried for preventing or managing diarrhea for your loved one over the past week? (Choose all that apply)
   - ☐ Using no constipation medications
   - ☐ Using a lower dose of constipation medications than before starting DAYBUE
   - ☐ Using antidiarrheal medications such as loperamide
   - ☐ Skipped dose(s) of DAYBUE
   - ☐ Did not take DAYBUE this week due to previous diarrhea
   - ☐ Consumed supplementary fiber (e.g., psyllium, wheat dextrin, flaxseed, etc.)
   - ☐ Specialized diet
   - ☐ Avoided sugar alcohols (e.g., sorbitol, mannitol) that are used to sweeten medications
   - ☐ Took a lower dose of DAYBUE than the target dose
   - ☐ Increased fluids to maintain hydration
   - ☐ Other (please describe in free text field below)
5. You selected Other above. Please describe. _____.

6. Over the **last 3 days**, has your loved one's stool been (Constipated; Formed/normal; Diarrhea)

[If Diarrhea is selected, respond to Questions 7-10. If not, proceed to Question 11]

7. Please pick the predominant form of diarrhea over the **last 3 days**. (Loose; Watery, contained inside the diaper; Watery, outside the diaper and on clothes; Outside the clothes [e.g., on chuck pads, wheelchair, bed])
8. Over the **last 24-hrs** how many diaper changes were required due to diarrhea? (0, 1, 2, 3, 4, 5, 6, 7, 8, >8)
9. Over the **last 24-hrs** how many toilet trips were required due to diarrhea? (0, 1, 2, 3, 4, 5, 6, 7, 8, >8)
10. Over the **last 24-hrs** how many clothing changes were required due to diarrhea? (0, 1, 2, 3, 4, 5, 6, 7, 8, >8)

Vomiting

11. Over the **last 24-hrs**, has your loved one experienced vomiting/retching/dry heaves? *[yes/no. If no, questionnaire is complete. If yes, continue to questions below]*

    Over the **last 24-hrs**, how many times did your loved one experience:

12. Vomiting
13. Regurgitation/spit-up
14. Retching/dry heaves
15. What appeared to trigger the vomiting? (Food, Beverage, DAYBUE, Other)
16. What appeared to trigger the regurgitation? (Food, Beverage, DAYBUE, Other)
17. Did you do any of the following to manage the vomiting over the past week?
    - ☐ Changed how loved one consumes things (amount, speed, etc.)
    - ☐ Changed diet
    - ☐ Took anti-vomiting/nausea medication
    - ☐ Changed loved one's body position
    - ☐ Other (please describe in free text field below)
18. You selected Other above. Please describe. _____.
19. Did you do any of the following to manage the regurgitation over the past week? Choose all that apply.
    - ☐ Changed how loved one consumes things (amount, speed, etc.)
    - ☐ Changed diet
    - ☐ Took anti-vomiting/nausea medication
    - ☐ Changed loved one's body position
    - ☐ Other (please describe in free text field below)
20. You selected Other above. Please describe. _____.

Abbreviation: GI=gastrointestinal.

RSBQ

The RSBQ is a 45-item rating scale completed by the caregiver that assesses a range of symptoms of Rett syndrome (breathing, hand movements or stereotypies, repetitive behaviors, night-time behaviors, vocalizations, facial expressions, eye gaze, and mood) (**Table 6**).[5,9] The RSBQ has been characterized and validated across a range of ages (2 to 47 years) and genetic variations in RTT.[10-14]

The caregiver rates each of the 45 items as "0" (not true), "1" (somewhat or sometimes true), or "2" (very true or often true). In general, the prompts for RSBQ represent symptoms of RTT, meaning that the score of "2" indicates greater severity or frequency of symptoms.[15] Lower scores reflect lesser severity in signs and symptoms of RTT.[9] The exception is item 31 ("Uses eye gaze to convey feelings, needs, and wishes") for which the score of "2" (very true) indicates a better outcome. In LAVENDER, the score for item 31 ("Uses eye gaze to convey feelings, needs, and wishes") was reversed in the calculations of total score because the score of "2" (very true) reflects a better outcome; therefore, decreases in RSBQ total score denote improvement.[15]

Table 6. RSBQ Items[5]

1. There are times when breathing is deep and fast (hyperventilation)
2. Spells of screaming for no apparent reason during the day
3. Makes repetitive hand movements with hands apart
4. Makes repetitive hand movements involving fingers around tongue
5. There are times when breath is held
6. Air or saliva is expelled from mouth with force
7. Spells of apparent anxiety/fear in unfamiliar situations
8. Grinds teeth
9. Seems frightened when there are sudden changes in own body position
10. There are times when parts of the body are held rigid
11. Shifts gaze with slow horizontal turn of head
12. Expressionless face
13. Spells of screaming for no apparent reason during the night
14. Abrupt changes in mood
15. There are certain days/periods where she performs much worse than usual
16. There are times when she appears miserable for no apparent reason
17. Seems to look through people into the distance
18. Does not use hands for purposeful grasping
19. Swallows air
20. Hand movements are uniform and monotonous
21. Has frequent naps during the day
22. Screams hysterically for long periods of time and cannot be consoled
23. Although can stand independently tends to lean on objects or people
24. Restricted repertoire of hand movement
25. Abdomen fills with air and sometimes feels hard
26. Spells of laughter for no apparent reason during the day
27. Has wounds on hands as a result of repetitive hand movements
28. Makes mouth grimaces
29. There are times when she is irritable for no apparent reason
30. Spells of inconsolable crying for no apparent reason during the day
31. Uses eye gaze to convey feelings, needs and wishes
32. Makes repetitive tongue movements
33. Rocks self when hands are prevented from moving
34. Makes grimacing expressions with face
35. Has difficulty in breaking/stopping hand stereotypies
36. Vocalizes for no apparent reason
37. Spells of laughter for no apparent reason during the night
38. Spells of apparent panic
39. Walks with stiff legs
40. Tendency to bring hands together in front of chin or chest
41. Rocks body repeatedly
42. Spells of inconsolable crying for no apparent reason during the night
43. The amount of time spent looking at objects is longer than the time spent holding or manipulating them
44. Appears isolated
45. Vacant ‘staring’ spells

Abbreviation: RSBQ=Rett Syndrome Behaviour Questionnaire.

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