TAKEDA PHARMACEUTICALS

PROTOCOL: TAK-555-4006

TITLE:	A Breast Milk Study in Lactating Women who Have Been Prescribed Therapeutic Doses of MOTEGRITY® (prucalopride) for Chronic Idiopathic Constipation to Evaluate Prucalopride Concentrations in Breast Milk, and to Collect Incidental Safety Data from the Nursing Infant
DRUG:	TAK-555, prucalopride succinate
IND:	55-078
EUDRACT NO.:	Non-EUDRACT
OTHER NO.:	NDA 210166
SPONSOR:	Takeda Development Center Americas, Inc. (TDC Americas) 95 Hayden Avenue Lexington, MA 02421
PRINCIPAL/ COORDINATING INVESTIGATOR:	[redacted], PhD, MPH [redacted]
PROTOCOL HISTORY:	Protocol V1.1 Amendment 1 January 26, 2021

PROTOCOL SIGNATURE PAGE

Sponsor's (Takeda) Approval

Signature: [redacted]	**Date:** 05-Feb-2021 | 23:36 JST
[redacted], M.S. [redacted], Safety Pharmacoepidemiology Global Evidence and Outcomes Takeda Pharmaceuticals	

Investigator's Acknowledgement

I have read this protocol for Study TAK-555-4006.

Title: *A Breast Milk Study in Lactating Women who Have Been Prescribed Therapeutic Doses of MOTEGRITY® (prucalopride) for Chronic Idiopathic Constipation to Evaluate Prucalopride Concentrations in Breast, and to Collect Incidental Safety Data from the Nursing Infant*

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a participant in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, participant to ethical and safety considerations and guidelines, and to conduct the study in accordance with International Council for Harmonisation guidelines of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address: (please hand print or type)	[redacted], PhD, MPH [redacted]

Signature: [redacted] **Date:** _____

EMERGENCY CONTACT INFORMATION

In the event of a Serious Adverse Event (SAE), the investigator must fax or e-mail the MedWatch form to the Global Patient Safety Evaluation (GPSE) Department. Requirements for SAE and AE reporting are specified in Appendix 5.3.2.

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ABBREVIATIONS

AE	adverse event
ASQ	Ages and Stages Questionnaire
AUC_{milk}	area under the milk concentration-time curve
CFR	Code of Federal Regulations
CIC	chronic idiopathic constipation
CRF	case report form
CRO	contract research organization
CV	coefficient of variation
EU	European Union
FDA	Food and Drug Administration
5-HT_4	5-hydroxytryptamine type 4
HMB	Human Milk Biorepository
IB	investigator's brochure
ICH	International Conference on Harmonisation
PK	pharmacokinetic(s)
SAE	serious adverse event
SAP	statistical analysis plan
τ	time interval over which AUC_{milk} was measured
UCSD	University of California, San Diego
US	United States

STUDY SYNOPSIS

Protocol number: TAK-555-4006	**Drug:** Prucalopride succinate
Title of the study: A Breast Milk Study in Lactating Women who Have Been Prescribed Therapeutic Doses of MOTEGRITY® (prucalopride) for Chronic Idiopathic Constipation to Evaluate Prucalopride Concentrations in Breast Milk, and to Collect Incidental Safety Data from the Nursing Infant	
Short title: An Observational, Milk Only Lactation Study in Breastfeeding Women who Have Been Prescribed MOTEGRITY	
Number of participants: 12	
Investigator(s): [redacted], PhD, MPH	
Site(s) and Region(s): One site; recruitment from residents of the United States (US)	
2020-2024	**Clinical phase:** 4

Objectives: The objectives of the study are to measure prucalopride concentrations in breast milk, and to describe adverse events (AEs), growth, and development of infants breastfed by mothers taking MOTEGRITY.

Primary:

- To quantify the concentration of prucalopride in breast milk over a 24-hour period of milk collection after a single dose following at least 5 consecutive daily doses.

Secondary:

- To describe the number and type of AEs reported in infants breastfed by mothers taking Motegrity (incidental safety reporting).
- To describe growth over the first year of life of infants breastfed by mothers taking MOTEGRITY.
- To describe performance on a development questionnaire in the first year of life in infants breastfed by mothers taking MOTEGRITY.

Rationale:

Chronic constipation is a highly prevalent condition estimated to affect as many as 14% of the population and is more common in women than men. MOTEGRITY is an oral selective serotonin (5-hydroxytryptamine type 4 [5-HT_4]) receptor agonist with enterokinetic properties that is currently approved in the US to treat chronic idiopathic constipation (CIC) under the trade name of MOTEGRITY.

MOTEGRITY is a gastrointestinal prokinetic agent that stimulates colonic peristalsis which increases bowel motility. Its plasma elimination half-life is estimated to be approximately 24 hours. The recommended dose of MOTEGRITY is 2 mg per day in patients without severe renal impairment (prescribing information [PI] for MOTEGRITY).

As CIC occurs in women of reproductive age who may be breastfeeding an infant, it is important to study the safety of MOTEGRITY in this population.

The current study is designed to satisfy a post marketing requirement (PMR 3529-5) as mandated in the new drug application (NDA) approval letter of December 2018, for MOTEGRITY (indicated for the treatment of CIC in adults).

Investigational product, dose, and mode of administration:

Participants will be taking MOTEGRITY, 2 mg oral tablets for functional constipation as per physician instructions.

Study Population:

The study population includes women, living in the US, who are currently being treated with MOTEGRITY for functional constipation and who are currently breastfeeding a single infant between the ages of 10 days and 11 months 0 days, inclusive, and who have not yet started to wean their child. Infants who are exclusively breastfed and do not yet eat solid foods are preferred.

Inclusion and exclusion criteria:

Inclusion Criteria:

Participants cannot be enrolled before all inclusion criteria are confirmed.

1. Female participants with an ability to voluntarily provide verbal followed by written, signed, and dated (personally or via a legally authorized representative) informed consent as applicable to participate in the study.
2. Healthy female participants as determined by the investigator on the basis of enrollment evaluations.
3. Participants ≥18 years of age at the time of consent. This inclusion criterion will only be assessed at the time of enrollment.
4. Participants who are currently breastfeeding a singleton infant who is between the ages of 10 days and 11 months 0 days, inclusive.
5. Participants who are currently exclusively breastfeeding or breastfeeding with supplemental formula and/or solid food. Infants who are exclusively breastfed and do not yet eat solid foods are preferred.
6. Participants who are currently treated as prescribed by their physician with MOTEGRITY for functional constipation for at least 5 consecutive days at the time of taking the first breast milk sample. All recommendations in the US PI should be followed.
7. Participants who agree to the conditions and requirements of the study including the sample collection, interview schedule, completion of development questionnaires, and release of medical records.
8. Participants with an understanding, ability, and willingness to fully comply with study procedures and restrictions.

Exclusion Criteria:

The participant will be excluded from the study if any of the following exclusion criteria are met:

1. Participants who are breastfeeding an infant who: is hospitalized, has a major birth defect, or has a history of a disease that could affect absorption or drug disposition.
2. Participants who have used MOTEGRITY while breastfeeding for a condition other than functional constipation.

3. Participants who are pregnant at the time of enrollment.
4. Participants who have started to wean their child from breast milk.
5. Participants with a history of any hematological, hepatic, respiratory, cardiovascular, renal, gall bladder removal, or other current or recurrent disease that could affect the action, absorption, or disposition of prucalopride.

Methodology:

Study Design:

This study will utilize a prospective, observational, exposure cohort design to recruit 12 breastfeeding women who are prescribed MOTEGRITY to treat functional constipation.

Consented women will provide breast milk samples from a single breast over a 24-hour period of collection using an electric breast pump, and drug levels in breast milk will be measured. Breast milk samples will be collected at home.

Breastfed infants will be enrolled between the ages of 10 days up to an age of 11 months 0 days, inclusive. They will be actively followed up to 12 months 30 days. During this Infant Follow-up Period, the infant's development will be monitored. In addition, the mother's breastfeeding status and current MOTEGRITY use will be documented. However, to allow for collection of questionnaires, medical records, etc, the maximum duration of participation of the mother-infant pair is expected to be approximately 16 months.

Setting:

The study will be conducted by investigators at the UC San Diego Human Milk Research Biorepository in the Department of Pediatrics School of Medicine at UC San Diego. Participants who meet eligibility criteria and who reside in the US will be recruited through referrals from the sponsor, health care providers, referrals from the Prucalopride Pregnancy Registry, the Mother-to-Baby network of counseling services, and direct to consumer awareness activities.

Study Size:

The target sample size is 12 mothers and their breastfeeding infants. This sample size will be evaluated for adequacy based on inter-individual variability when the first 6 participants' milk samples have been analyzed.

Duration of the Study:

The study is planned for 4 years from the enrollment of the first participant until study completion. This will include 3 years of active recruitment, as shown in Table 1, with an annual status report provided to the sponsor each year. The final report with descriptive analysis according to the statistical analysis plan (SAP) will be prepared at the end of the study.

Table 1 Anticipated Recruitment Timetable and Sample Size

Year 1	Year 2	Year 3
Enroll 4 mothers and infants	**Enroll** 4 mothers and infants	**Enroll** 4 mothers and infants

The maximum duration of participation of the mother-infant pair is expected to be approximately 16 months to allow for collection of questionnaire and medical records data.

Variables:

Exposure will be defined as MOTEGRITY treatment by maternal report, verified by medical record review, with dose and dates of exposure.

Outcome variables will include:

Quantification of prucalopride concentration in breast milk samples taken over a 24-hour collection period. Prucalopride concentration values will be used to determine the area under the concentration-time curve (AUC). The average milk concentration will be calculated from prucalopride's AUC in milk as per the following equation:

$$Average\ concentration\ in\ milk = \frac{AUC}{Time\ interval\ over\ which\ the\ AUC\ was\ measured}$$

Infant outcomes (growth and performance on developmental screening) up to 12 months 30 days of adjusted infant age.

Infant outcomes (consisting of a pre-defined list of adverse events (AEs) as well as an open-ended question about any additional AEs) to 12 months 30 days of adjusted age including maternal report of AEs in the infant while being breastfed by a mother who is taking Motegrity.

Infant outcomes will be obtained by maternal report (by phone), medical records abstraction, and maternal responses to neurodevelopmental screening questionnaires. Covariates to be collected regarding the mother include age, race/ethnic group, socioeconomic status, body mass index (BMI), medical history, breastfeeding history, current feeding patterns with breast milk, formula, and solid food, and other exposures while breastfeeding. Variables to be collected regarding the infant include age and sex of the infant, gestational age at birth, birth size (length and weight), and comorbidities.

Data Sources:

Data for the primary objective will be obtained from one 24-hour breast milk sample collection and subsequent assay of the samples collected. Data for the secondary objectives will be obtained through maternal interview(s), maternally completed developmental screening questionnaires, and medical record abstraction.

Data Analysis:

The quantification of levels of prucalopride in breast milk will be determined using a validated method to determine drug levels. Standard approaches will be used to estimate infant consumption based on breastfeeding habits. Data analysis for the secondary objectives will be descriptive. Means, standard deviations (SDs), and 95% CI will be presented for continuous variables and frequencies and percentages will be presented for categorical variables. Associations will be explored between measurable levels of drug in breast milk and infant outcomes.

Data Protection:

All data for the Human Milk Biorepository at UC San Diego is securely stored in a customized version of a Clinical Trials Management database, Velos, supported by UC San Diego Health Sciences with password protection and restricted access on secure servers. Personal identifying information including participant name, date of birth, sex, are securely stored in the database. Each participant is also assigned a unique participant ID

(PID) number. All biospecimen, assay measures, interview, medical record abstraction and questionnaire data are stored by PID number in the database. Biospecimens are identified only by PID number.

The primary investigator will provide appropriate permission to the designated study staff who will manage all data, biospecimens, regulate distribution of specimens, and relevant study data. Hard copy forms of study data collection instruments and informed consent documents are to be maintained in locked file cabinets with restricted access at the study site.

All sample distributions will go to the commercial laboratory selected by the Sponsor. For sample distribution, the designated study coordinator will use the PID to generate a list of the Biospecimen Tube ID numbers eligible for the analysis. Selected samples are identified by Tube ID and associated sample collection characteristics, such as time of collection.

Results from the commercial laboratory will be returned to the study PI and sent into the secure database by Tube ID and PID.

Data Security Procedures:

Signed consent forms and hard copy files that contain personal identifying information collected from participants are kept in locked files; password protected and restricted access computer files on secure servers in restricted access offices.

The study database is maintained in the UC San Diego Health Sciences IT environment with appropriate protection.

Maximum duration of participant involvement in the study:

The maximum duration of participation in the study for a participant and her breastfed infant is approximately 16 months to allow for final collection of data from questionnaires and medical records.

Sample Collection and Handling Procedures:

Sample Collection: A series of 7 timed, full expression breast milk samples will be collected from a single breast using an electric breast pump over one 24-hour period for breast milk prucalopride assays. No additional samples will be collected.

The breast milk sample collection schedule will be as follows in relation to the time the mother takes her daily dose of MOTEGRITY: Time 1 (pre-dose), Time 2 (1 hour post-dose), Time 3 (2 hours post-dose), Time 4 (4 hours post-dose), Time 5 (8 hours post-dose), Time 6 (12 hours post-dose), and Time 7 (24 hours post-dose). Participants will extract a portion of each milk expression for the study sample collection.

Sample Handling: Participating mothers will record the quantity of each full expression, extract a small sample of milk (approximately 10 mL) from each expression, and refrigerate until shipment back to the study center.

Once samples have been received by the study staff, samples will be processed according to laboratory manual requirements until shipment to the commercial laboratory selected by the sponsor for analysis.

Endpoints and statistical analysis:

Analysis populations:

- The Enrolled Set will consist of all participants who have provided informed consent and meet inclusion/exclusion criteria.
- The Safety Set will consist of all participants who provide a breast milk sample.

- The Pharmacokinetic Set will consist of all participants in the Safety Set for whom at least 4 PK samples (pre-dose, 4, 12, and 24 hours post-dose) are evaluable.

Study endpoints:

Primary: Prucalopride concentration in breast milk.
Secondary: Maternal report of infant AEs, growth, and performance on development questionnaires.

Statistical methodology for primary endpoints:

All relevant prucalopride concentration data will be summarized using descriptive statistics. The N, mean, SD, 95% CI, median, and range will be reported for continuous variables.

Statistical methodology for secondary endpoints:

All endpoints will be summarized using descriptive statistics. The N, mean, SD, 95% CI, median, and range will be presented for continuous variables, and frequencies and percentages will be presented for categorical variables. Associations between maternal drug levels and infant outcomes will be explored.

Sample size justification:

It is expected that approximately 12 female participants will enroll into this study with complete sample collection data. Sample size specified within the Food and Drug Agency's guideline range of 8 to 20 participants. As the inter-participant variability in the pharmacokinetics of prucalopride in breastmilk is unknown, an interim assessment of appropriateness of the sample size is planned. After the first 6 participants' samples have been assayed, the inter-participant variability will be evaluated to determine if an increased sample size is necessary and feasible (details to be provided in the SAP).

Additional details will be provided in the SAP.

STUDY SCHEDULE(S)

Table 2 Schedule of Assessments

Visit	Enrollment Period	Breast Milk Sampling Period		Infant Follow-up Period/EOS
Study Day	**-14 to -2**	**-1**	**1**	**Bi-monthly[a]**
Screening and Prescreening Assessments				
Informed consent	X			
Enrollment	X			
Request for medical records and medical record abstraction (mother and infant)	X		X	X
Inclusion/exclusion criteria	X	X		
Confirm 5 consecutive days of MOTEGRITY dosing at the time of the first breast milk sample		X		
Safety Assessments				
AE/SAE (infant)	X	X	X	X
Pharmacokinetic Assessments				
Breast milk sampling[b,c]			X	
Other Assessments				
ASQ[d]	X		X	X
Enrollment/interim interview[d,e]	X		X	X

AE=adverse event; ASQ= Ages and Stages Questionnaire; BMI=body mass index; EOS=end of study; SAE=serious adverse event

[a] Bi-monthly up to 12 months 30 days of adjusted infant age.

[b] Breast milk samples (aliquots) will be taken pre-dose and at 1, 2, 4, 8, 12, and 24 hours following the mother's morning dose of MOTEGRITY. It should be noted that since no samples should arrive at the bioanalytical laboratory on the weekend, they should be shipped Monday-Wednesday only. This implies that the start of the sample collection has to be on a Sunday, Monday, or Tuesday.

[c] Mothers will record the quantity of each full expression, extract a small sample of milk (~10 mL) from each expression, and refrigerate until shipment back to the study center.

[d] Maternal report.

[e] Including questions on demography, medical/medication history, breastfeeding/pregnancy status and current MOTEGRITY use. Specific information to be collected regarding the mother includes age, race/ethnic group, socioeconomic status; BMI, medical history, pregnancy status, breastfeeding history, current feeding patterns with breast milk and formula, and other exposures while breastfeeding. Information to be collected regarding the infant includes age and sex of the infant, gestational age at birth, birth size, and comorbidities.

1. BACKGROUND INFORMATION

1.1 Indication and Current Treatment Options

Prucalopride is a highly selective 5-hydroxytryptamine type 4 (5-HT_4) receptor agonist that stimulates colonic motility to provide effective relief to patients with chronic idiopathic constipation (CIC). Chronic idiopathic constipation is characterized by symptoms including straining during defecation, lumpy or hard stools, sensation of incomplete evacuation, and fewer than 3 defecations per week. Prucalopride exerts its effect in CIC by increasing the number and amplitude of high amplitude propagating contractions, ie, the peristaltic contractions moving the contents of the colon forward. An estimated 35 million adults have CIC in the United States (US) (Suares and Ford, 2011).

Chronic idiopathic constipation is more prevalent in women, non-Whites, and elderly patients. Treatment interventions for CIC range from lifestyle modifications to prescription therapies; however, there continues to be high patient dissatisfaction with available therapies. Treatment for CIC typically begins with diet changes (increase in dietary fiber), increasing fluid intake, and exercise. Patients then progress to over-the-counter osmotic laxatives (ie, polyethylene glycol), bulking agents, stool softeners, or stimulants, all of which have limited effectiveness. Current prescription therapies for CIC – lubiprostone, linaclotide, and plecanatide – function via increasing colonic secretions (ie, prosecretory) but do not have a direct effect on colonic peristalsis. However, due to the similar mechanism of action of these prosecretory agents, patients who do not respond have no alternative treatment options. As CIC occurs in women of reproductive age who may be breastfeeding an infant, it is important to study the safety of MOTEGRITY in this population.

1.2 Product Background and Clinical Information

Prucalopride (also referenced as SPD555, SHP555, M0001, R093877, and R108512 in countries where the drug is approved as MOTEGRITY®, RESOLOR®, RESOTRAN®, RESOTRANS®, and RESOLOR™) is a drug that stimulates gastrointestinal motility. The drug is approved in the US for adult patients with chronic idiopathic constipation
(Takeda Pharmaceuticals Company Limited, 2020).

Prucalopride was first approved in the European Union (EU) in October 2009 (centralized procedure) for use in women only, and approval for use in both adult men and women was received in June 2015. Prucalopride has been granted a marketing authorization in 83 countries worldwide and is currently marketed in 60 countries. Cumulatively through to October 2018, the estimated worldwide patient exposure to prucalopride is 346,069 person-years treatment.

Prucalopride belongs to a chemical class of dihydrobenzofuran-carboxamide derivatives with strong enterokinetic activity. In fasted awake dogs, the compound induces giant migrating contractions, stimulates proximal colonic motility, enhances gastro-pyloric-duodenal motility, and accelerates gastric emptying (Briejer et al., 1997b; Wellens and Schuurkes, 1996).

It is the first selective high affinity 5-HT_4 receptor agonist, which is likely to explain its enterokinetic effects in dogs as well as in humans (Briejer et al., 1995; Briejer et al., 1997b;

Briejer et al., 1997a). Prucalopride is highly selective when compared to other drugs with 5-HT_4 receptor agonistic properties: tegaserod and cisapride are non-selective 5-HT_4 receptor agonists that interact with other receptors in a concentration range relevant for their interaction with 5-HT_4 receptors.

The most accurate and current information regarding the drug metabolism, pharmacokinetics (PK), efficacy, and safety of prucalopride as well as the overall benefit/risk assessment can be found in the investigator's brochure (IB) and the current prescribing information (Takeda Pharmaceuticals Company Limited, 2020).

1.3 Risk/Benefit and Ethical Assessment

Prucalopride benefits/risk profile remains favorable and provides a treatment option for patients with chronic constipation.

The safety measures in this study are considered standard for a milk-only lactation study in a nursing mother who is taking MOTEGRITY for functional constipation and for the breast fed infant of that nursing mother. These include signed informed consent, evaluation of medical history, physical examination, and adverse events (AEs) for the nursing mother, and infant outcomes up to 12 months 30 days infant age including maternal report of AEs in the infant while being breastfed by a mother who is taking prucalopride, infant growth, and performance on development.

1.4 Compliance Statement

This study will be conducted in accordance with this protocol, the International Conference on Harmonisation Guideline for Good Clinical Practice E6 (ICH GCP, 1996; E6 R2, 2017), Title 21 of the US Code of Federal Regulations (US CFR).

The responsibilities of the study sponsor and investigator(s) are described fully in Appendix 4.

2. STUDY OBJECTIVES AND PURPOSE

2.1 Rationale for the Study

Chronic constipation is a highly prevalent condition estimated to affect as many as 14% of the population and is more common in women than men (Suares and Ford, 2011; Camilleri et al., 2017). Prucalopride is an oral selective serotonin (5-HT_4) receptor agonist with enterokinetic properties that is currently approved in the US to treat CIC.

A study evaluating the secretion of prucalopride into breast milk (PRU-RSA-1) in 8 healthy lactating females no longer breastfeeding their babies or lactating females agreeing to stop breastfeeding before the first intake of prucalopride demonstrated that prucalopride is excreted in breast milk. The average daily amount passed to the infant is estimated to be 1.7 µg/kg. Adjusted for body weight, this represents about 6% of the maternal dose, indicating that prucalopride exposure is about 16 times lower in the infant than in the mother. As CIC occurs in women of reproductive age who may be breastfeeding an infant, it is important to evaluate the safety of MOTEGRITY in this population.

The current study is designed to satisfy a post marketing requirement (PMR 3529-5) as mandated in the new drug application (NDA) approval letter of December 2018, for prucalopride (indicated for the treatment of CIC in adults) (Takeda Pharmaceuticals Company Limited, 2020).

2.2 Objectives

The objectives of the study are to measure prucalopride concentrations in breast milk, and to describe adverse events (AEs), growth and development of infants breastfed by mothers taking MOTEGRITY.

2.2.1 Primary Objectives

- To quantify the concentration of prucalopride in breast milk over a 24-hour period of milk collection after a single dose following at least 5 consecutive daily doses.

2.2.2 Secondary Objectives

- To describe the number and type of AEs reported in infants breastfed by mothers taking Motegrity (incidental safety reporting).
- To describe growth over the first year of life of infants breastfed by mothers taking Motegrity.
- To describe performance on a development questionnaire in the first year of life in infants breastfed by mothers taking Motegrity.

3. STUDY DESIGN AND FLOW CHART

3.1 Study Design and Flow Chart

This study will utilize a prospective, observational, exposure cohort design to recruit 12 breastfeeding women who are prescribed MOTEGRITY to treat functional constipation.

The study will consist of an Enrollment Period, a Breast Milk Sampling Period, and an Infant Follow-up Period.

After providing informed consent, women will provide breast milk samples from a single breast over a single breast over a 24-hour Breast Milk Sampling Period using an electric breast pump. Breast milk samples will be taken at home.

Prucalopride levels in breast milk will be measured. Participants who are not able to complete the minimum sample collection (at pre-dose, 4 hours, 12 hours, and 24 hours post-dose) will be given the opportunity to supply a new set of samples. Additional information on sample collection and handling is provided in Section 7.1.3 and the laboratory manual.

Breastfed infants will be enrolled up to an age of 11 months 0 days. They will be actively followed up to 12 months 30 days. During this Infant Follow-up Period, the infant's development will be monitored. In addition, the mother's breastfeeding status and current MOTEGRITY use will be documented. To allow for collection of questionnaires, medical records, etc, the maximum duration of participation of the mother-infant pair is expected to be approximately 16 months.

An overview of the timing of enrollment, breast milk sampling, and other assessments is provided in Table 2.

3.2 Study Size

The target sample size is 12 breastfeeding mothers and their infants. As the inter-participant variability in prucalopride concentrations in milk is unknown, an interim assessment is planned. After the first 6 participants' samples have been assayed the inter-participant variability will be evaluated to determine if an increased sample size is necessary and feasible (details provided in the SAP).

3.3 Duration and Study Completion Definition

The maximum duration of participation of the mother-infant pair is expected to be approximately 16 months to allow for collection of questionnaire and medical records data.

The Study Completion Date is defined as the date on which the last participant, in the study completes the final protocol-defined assessment(s). Please note that this includes the follow-up visit or contact, whichever is later (refer to Section 7.1.3 for the defined follow-up period for this protocol).

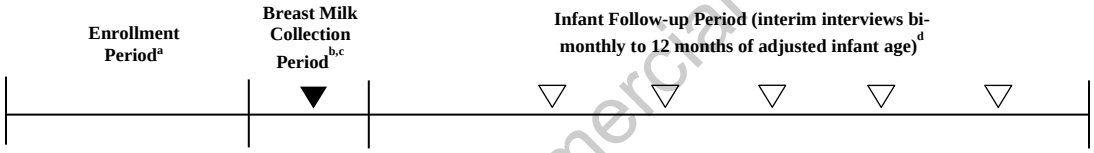
The study is planned for 4 years from the enrollment of the first patient until study completion. There will be 3 years of active recruitment, as shown in Table 3. Recruitment rate will be assessed at the interim analysis, and modifications made to study duration and/or recruitment strategies will be determined. An annual status report provided to the sponsor each year. The final report with descriptive analysis according to the statistical analysis plan (SAP) will be prepared at the end of the study.

Table 3 Anticipated Recruitment Timetable and Sample Size

Year 1	Year 2	Year 3
Enroll 4 mothers and infants	**Enroll** 4 mothers and infants	**Enroll** 4 mothers and infants

A flow chart of the study is provided in Figure 1.

Figure 1 Study Design Flow Chart



[a] Enrollment (intake/enrollment interview) and consent.

[b] Exposure will be defined as MOTEGRITY treatment by maternal report, verified by medical record review, with dose and dates of exposure.

[c] A series of 7 timed breast milk samples will be collected from a single breast using an electric breast pump over a 24-hour period for breast milk prucalopride assay. Breast milk samples will be collected pre-dose and for 24 hours post-dose once.

[d] Infant outcomes including maternal report of adverse events in the infant, infant growth, and performance on development questionnaires up to 12 months 30 days of adjusted infant age. To allow for collection of questionnaires, medical records, etc, the maximum duration of participation of the mother-infant pair is expected to be approximately 16 months.

3.4 Sites and Regions

The study will be conducted by investigators at the UC San Diego Human Milk Research Biorepository in the Department of Pediatrics School of Medicine at UC San Diego. Participants who meet eligibility criteria and who reside in the US will be recruited through referrals from the sponsor, health care providers, referrals from the Prucalopride Pregnancy Registry, the Mother-to-Baby network of counseling services, and direct to consumer awareness activities. The study will be conducted at 1site, and will involve recruitment of approximately 12 women residing anywhere in the US.

4. STUDY POPULATION

Each participant must engage in the informed consent process. Initial enrollment will be completed with verbal consent administered by telephone to allow for collection of interview data. Written informed consent will be provided at the time of milk collection and shipped back to the Study Center with the milk samples.

4.1 Inclusion Criteria

The participant will not be considered eligible for the study without meeting all of the criteria below.

Participants cannot be enrolled before all inclusion criteria are confirmed.

1. Female participants with an ability to voluntarily provide verbal followed by written, signed, and dated (personally or via a legally authorized representative) informed consent as applicable to participate in the study.

2. Healthy female participants as determined by the investigator on the basis of enrollment evaluations.

3. Participants ≥18 years of age at the time of consent. This inclusion criterion will only be assessed at the time of enrollment.

4. Participants who are currently breastfeeding a singleton infant who is between 10 days and 11 months 0 days.

5. Participants who are currently exclusively breastfeeding or breastfeeding with supplemental formula and/or solid food. Infants who are exclusively breastfed and do not eat solid food are preferred.

6. Participants who are currently treated as prescribed by their physician with MOTEGRITY for functional constipation for at least 5 consecutive days at the time of taking the first breastmilk sample. All recommendations in the US PI should be followed.

7. Participants who agree to the conditions and requirements of the study including the sample collection, interview schedule, completion of developmental questionnaires, and release of medical records.

8. Participants with an understanding, ability, and willingness to fully comply with study procedures and restrictions.

4.2 Exclusion Criteria

The participant will be excluded from the study if any of the following exclusion criteria are met.

1. Participants who are breastfeeding an infant who: is hospitalized, has a major birth defect, or has a history of a disease that could affect absorption or drug disposition.

2. Participants who have used MOTEGRITY while breastfeeding for a condition other than functional constipation.

3. Participants who are pregnant at the time of enrollment.

4. Participants who have started to wean their child from breast milk.

5. Participants with a history of any hematological, hepatic, respiratory, cardiovascular, renal, gall bladder removal, or current or recurrent disease that could affect the action, absorption, or disposition of prucalopride.

4.3 Reproductive Potential

Female participants who are known to be pregnant at the time of enrollment will be excluded. Pregnancy status will be assessed at each subsequent maternal interview until study participation is completed.

4.4 Discontinuation of Participants

A participant may withdraw from the study at any time for any reason without prejudice to his/her future medical care by the physician or at the institution, or may be withdrawn at any time at the discretion of the investigator or sponsor.

Participants who discontinue from the study may be replaced at the sponsor's discretion to ensure that at least 12 participants complete the study.

4.4.1 Reasons for Discontinuation

The reason for discontinuation must be determined by the investigator and recorded in the participant's source document. If a participant discontinued for more than 1 reason, each reason should be documented in the source document and the most clinically relevant reason should be indicated.

Reasons for discontinuation include but are not limited to:

1. Withdrawal by participant.

2. Lost to follow-up.

3. Other.

4.4.2 Participants 'Lost to Follow-up' Prior to Last Scheduled Visit

A minimum of 3 attempts must be made to contact any participant lost to follow-up at any time point prior to the last scheduled contact and documented.

5. PRIOR AND CONCOMITANT TREATMENT

5.1 Prior Treatment and Other Relevant Exposures

Information on dates, dosages, and indications for prior treatment will be collected by maternal interview. Prior treatment includes prescription medications, over-the-counter medications, herbal treatments, vitamins, caffeine, vaccines, and other substances in the previous 2 weeks prior to the milk sampling date and at each subsequent bi-monthly interview as long as the mother continues to breastfeed.

5.2 Concomitant Treatment

Information on dates, dosages, and indications for concomitant treatment will be collected by maternal interview. Concomitant treatment includes prescription medications, over-the-counter medications, herbal treatments, vitamins, caffeine, vaccines, and other substances in the previous 2 weeks prior to the milk sampling date and at each subsequent bi-monthly interview as long as the mother continues to breastfeed.

5.2.1 Permitted Treatment

There are no known clinically significant interactions with MOTEGRITY and other drugs; therefore, this study does not restrict prior and concomitant medication use.

6. INVESTIGATIONAL PRODUCT

6.1 Identity of Investigational Product

Not applicable. This is a noninterventional study.

6.1.1 Blinding the Treatment Assignment

Not applicable. This is a noninterventional study.

6.1.2 Prescribed Investigational Product

MOTEGRITY® (prucalopride succinate), 2-mg oral tablets will have been prescribed and treatment initiated prior to enrollment.

6.2 Administration of Investigational Product(s)

6.2.1 Allocation of Participants to Treatment

Not applicable. This is a noninterventional study

Participant numbers are assigned to all participants as they consent to take part in the study.

An 8-digit participant number is randomly assigned at the time of enrollment, and is not used again for any other participant.

6.2.2 Dosing

Participants will have begun and are maintaining MOTEGRITY treatment as prescribed and directed by their physician.

7. STUDY PROCEDURES

7.1 Study Schedule

Refer to Table 2 for an overview of the timing of enrollment, breast milk sampling, and other assessments.

7.1.1 Enrollment Period (Day -14 to Day -2)

At the time of enrollment, eligible participants will complete a verbal consent administered by telephone in order for the initial interview data to be collected.

Written, signed, and dated informed consent will be obtained from the participant at the time of milk collection and mailed to the site with the milk samples. A copy of the written informed consent will be provided to the participant with the milk collection materials.

7.1.2 Breast Milk Sampling Period

A series of complete breast milk collections from a single breast will be obtained using an electric breast pump at 7 designated times over a 24-hour period at the time points indicated in Table 2 and below:

The breast milk sample collection schedule in relation to the time the mother takes her daily dose of MOTEGRITY will be as follows: Time 1 (pre-dose), Time 2 (1 hour post-dose), Time 3 (2 hours post-dose), Time 4 (4 hours post-dose), Time 5 (8 hours post-dose), Time 6 (12 hours post-dose), and Time 7 (24 hours post-dose). Participants will extract a portion of each milk expression for the study sample collection and the remaining breast milk can be used for infant feeding if the mother chooses to do so.

7.1.3 Infant Follow-up Period

At the time points indicated in Table 2, maternal report of the infant breastfed by a mother who is or was taking MOTEGRITY, including incidence of AEs, infant growth, and development up to 12 months 30 days, will take place.

Infant outcomes (infant growth, development, and toxicities [based on a predefined list of adverse reactions as well as an open ended AE question]) will be obtained by maternal report, medical records abstraction, and maternal responses to a development questionnaire.

In addition, the mother's breastfeeding status and current MOTEGRITY use will be documented bimonthly.

7.1.4 Additional Care of Participants After the Study

No after care is planned for this study.

7.2 Study Evaluations and Procedures

7.2.1 Safety

The name and address of each third party vendor used in this study will be maintained in the investigator's and sponsor's files.

7.2.1.1 Adverse Event Questionnaire (Infant)

At each study interview, participants who continue to breastfeed their infant will be asked to respond to a predefined checklist of AEs occurring in their infant over the previous 2 weeks. An open-ended AE question will also be asked at each study interview to record any event that was not reported on the predefined checklist. Adverse event collection and reporting is discussed in Appendix 5.

7.2.2 Pharmacokinetic Analysis

7.2.2.1 Breast Milk Sample Collection and Handling Procedures

Breast milk samples will be collected at the times specified in Table 2 to measure breast milk prucalopride concentrations.

Milk samples (approximately 10 mL aliquot each) will be taken from the breast milk expressions and will be immediately refrigerated. The actual time that the sample was obtained and the volume collected will be recorded by the participant on a form provided with the milk collection materials. All milk samples along with the corresponding documentation will be packaged with cooling packs and shipped to UC San Diego study center. Once refrigerated samples are received at the UC San Diego study center, samples will be split into 5 of ~2-mL aliquots and frozen. Two frozen aliquots will be shipped to the PPD bioanalytical lab for PK analysis. The remaining 3 aliquots will be retained in the UC San Diego biorepository.

The details of milk sample collections, storage, and handling will be described in the study lab manual.

7.2.2.2 Shipment of Breast Milk Samples

The details of milk samples storage and shipment conditions will be described in the study lab manual. Shipments should be scheduled so that no samples arrive on the weekend and should be shipped Monday to Wednesday only. Samples should be transported to ensure that they arrive at the designated bioanalytical laboratory between the hours of 9:00 AM and 4:00 PM. The recipient and primary sponsor contact must be notified by telephone or e-mail when the samples are shipped, and they must be provided with the shipment tracking number.

All breast milk samples, along with the corresponding documentation, will be shipped to the University of California, San Diego
(UCSD) study center:

Attention: [redacted]
University of California, San Diego
7910 Frost St, Suite 370

San Diego, CA 92123
Telephone: [redacted]
Fax: [redacted]
Email: [redacted]

Two aliquots of PK samples, along with the corresponding documentation, will be shipped from UCSD to PPD bioanalytical lab frozen on dry ice:

Attention: Sample Management Department
PPD
3230 Deming Way
Middleton, WI 53562
Telephone: [redacted]
Email: [redacted]

7.2.2.3 Breast Milk Drug Concentration Assay Methodology

Breast milk PK samples will be analyzed for drug concentrations at the PPD bioanalytical lab using a validated method. The detailed method information will be described in the validation report. Drug concentrations will be reported to the study center. Raw data will be archived at PPD.

7.2.3 Other Assessments

7.2.3.1 Enrollment and Interim Interview

At the time points indicated in Table 2, the mother will complete an enrollment/interim interview document.

Information to be collected regarding the mother includes age, race/ethnic group, socioeconomic status, body mass index (BMI), breastfeeding history, current feeding patterns with breast milk and formula, and medical/medication history. Information to be collected regarding the breastfeeding infant includes age and sex of the infant, gestational age at birth, birth size, and medical/medication history.

The enrollment/interim interviews are provided in Appendix 2.

7.2.3.2 Medical Records

At the time points indicated in Table 2, the mother/infants' medical records will be requested and abstracted.

7.2.3.3 Ages and Stages Questionnaire

The Ages and Stages questionnaire (ASQ) will be completed by the mother at the time points indicated in Table 2.

The ASQ is a parent-completed questionnaire that will be used as a general developmental screening tool. The ASQ-3 is a parent reported initial level developmental screening instrument

consisting of 21 intervals, each with 30 items in 5 areas: (i) personal social, (ii) gross motor, (iii) fine motor, (iv) problem solving, and (v) communication for children from 2-66 months.

The ASQ is provided in Appendix 3.

8. DATA MANAGEMENT AND STATISTICAL METHODS

8.1 Data Collection

The investigators' authorized site personnel will enter data collected from maternal interviews, abstracted medical records, and obtained from developmental questionnaires into the Human Milk Biorepository Database. Results of sample assays once received from the Bioanalytical Laboratory will also be entered into the study database.

8.2 Clinical Data Management

According to study standard operating procedures, all data collected for the study is validated and double-entered to confirm accuracy.

8.3 Data Acquisition

Data for the primary objective will be obtained from one 24-hour breast milk sample collection and subsequent assay of the samples collected. Data for the secondary objectives will be obtained through maternal interview(s), maternally completed development questionnaires, and medical record abstraction.

8.4 Statistical Analysis Process

All PK analyses will be completed by investigators in the bioanalytical lab at UCSD based on assay data generated by PPD.

The SAP will provide the statistical methods and definitions for the analysis of the safety and breast milk data, as well as describe the approaches to be taken for summarizing other study information such as participant disposition, demographics, and concomitant medications. The SAP will include a description of how missing, unused and spurious data will be addressed. The SAP will also describe the methods for conducting an interim assessment of the adequacy of the target sample size based on inter-participant variability.

The SAP will be finalized within 30 days following final draft of study protocol to preserve the integrity of the statistical analysis and study conclusions.

All statistical analyses will be performed using R® Version 3.5.3 or higher.

8.5 Planned Interim Analysis, Adaptive Design, and Data Monitoring Committee

Inter-participant variability will be assessed after the first 6 participants' milk samples have been assayed to determine if the sample size is adequate. The interim status report that will be sent to the FDA will not include an analysis of available data, but will provide justification for the final study sample size. It will only be a status report to ensure on-time enrollment and the availability of the clinical study report. However, the results of the interim assessment of sample variability will be provided to the FDA with the final status report.

8.6 Sample Size Calculation and Power Considerations

Sample size was based on feasibility considerations as no formal inferential statistical analysis is planned for this study. The chosen sample size of 12 participants is considered adequate based on the following recommendation of the FDA guidance below (FDA, 2005). However, an interim analysis regarding inter-participant variability with respect to the planned sample size is planned as described in Section 8.5.

A sample size of 8 to 20 participants has been utilized in milk only lactation studies, which is supported by the Food and Drug Administration (FDA)'s Guidance document which states "Since milk only studies are more exploratory in nature, a minimum of 6 to 8 participants can be sufficient." (FDA, 2005)

8.7 Analysis Populations

The **Enrolled Set** will consist of all participants who have provided informed consent and meet inclusion/exclusion criteria.

The **Safety Set** will consist of all participants who provide a breast milk sample.

The **Pharmacokinetic Set** will consist of all participants in the Safety Set for whom at least 4 PK samples (pre-dose, 4, 12, and 24 hours post-dose) are evaluable.

8.8 Statistical Methodology for Primary Endpoints

8.8.1 Definition of Primary Endpoint

The primary endpoint is prucalopride concentration in breast milk.

8.8.2 Description of Primary Endpoint (Pharmacokinetic) Parameters

All PK analyses will be performed using the Pharmacokinetic Set.

Pharmacokinetic parameters will be determined from the breast milk concentration-time data for prucalopride by noncompartmental analysis using actual sampling times. The PK parameters will include, but not be limited to:

- AUC_{milk}: area under the milk concentration-time curve
- average concentration in milk
- τ: the time interval over which the AUC_{milk} was measured

The drug concentrations in the breast milk samples are used to calculate an area under the milk concentration-time curve (AUC_{milk}) (Anderson, 2018; Begg et al., 2002; FDA, 2005):

$$Average\ concentration\ in\ milk = \frac{AUC}{T}$$

Daily Infant Dosage (mg/kg/day) equals the product of the average concentration of drug excreted in milk and the total volume of milk consumed by the infant per kg per day.

The weight-adjusted percentage of the maternal dose (also called the relative infant dosage [RID]) consumed in breast milk over 24 hours will be calculated:

$$\%\, maternal\ dosage = \frac{Infant\ dosage\ (\frac{\frac{mg}{kg}}{day})}{Maternal\ dosage\ (\frac{\frac{mg}{kg}}{day})}\ x\ 100$$

8.8.3 Statistical Analysis of Primary Endpoint

There will be no inferential statistical analysis of the PK data. Summary statistics (number of observations, mean, SD, 95% CI, coefficient of variation [CV%], median, maximum, minimum, geometric mean, and CV% of geometric mean) will be determined for all PK parameters. Breast milk concentrations at each nominal sampling time will also be summarized using descriptive statistics.

The primary PK endpoints are: average prucalopride concentration in breast milk, and AUC_{milk}. The secondary PK endpoints are daily infant dosage, and % maternal dosage. .

8.9 Statistical Methodology for Secondary Endpoints

8.9.1 Definition of Secondary Endpoints

The secondary endpoints of the study are maternal report of infant AEs, growth and performance on development questionnaires.

8.9.2 Statistical Analysis of Secondary Endpoints

All endpoints will be summarized using descriptive statistics. Means and SDs will be presented for continuous variables and frequencies and percentages will be presented for categorical variables. Associations between drug levels and infant outcomes will be explored.

8.10 Other Analyses

No other analyses are planned in this study.

9. REFERENCES

Anderson, P. O. 2018. Drugs in Lactation. *Pharm Res,* 35, 45.

Begg, E. J., Duffull, S. B., Hackett, L. P. & Ilett, K. F. 2002. Studying drugs in human milk: time to unify the approach. *J Hum Lact,* 18, 323-32.

Briejer, M., Ghoos, E., Eelen, J. & Schuurkes, J. 1997a. Study on the involvement of serotonin 5-HT4 receptors in the effects of R093877 on colonic motility patterns in fasted conscious dogs. *Janssen Research Foundation Preclinical Research Report.*

Briejer, M., Van Bergen, P., Borgmans, C., De Winter, P. & Schuurkes, J. 1997b. R093877 is a selective and relatively potent 5-HT4 receptor agonist in guinea-pig and rat intestinal tissues. *Janssen Research Foundation Preclinical Research Report.*

Briejer, M. R., Akkermans, L. M. & Schuurkes, J. A. 1995. Gastrointestinal prokinetic benzamides: the pharmacology underlying stimulation of motility. *Pharmacological Reviews,* 47, 631-51.

Camilleri, M., Ford, A. C., Mawe, G. M., Dinning, P. G., Rao, S. S., Chey, W. D., Simren, M., Lembo, A., Young-Fadok, T. M. & Chang, L. 2017. Chronic constipation. *Nat Rev Dis Primers,* 3, 17095.

EMA. 2017. *Guideline on good pharmacovigilance practices (GVP): Module VI—collection, management and submission of reports of suspected adverse reactions to medicinal products [EMA/873138/2011 Rev 2]* [Online]. Available: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/guideline-good-pharmacovigilance-practices-gvp-module-vi-collection-management-submission-reports_en.pdf [Accessed].

FDA 2005. Guidance for Industry: Clinical Lactation Studies – Study Design, Data Analysis, and Recommendations for Labeling, February, 2005.

FDA. 2018. *Motegrity NDA 210166 approval letter, December 14, 2018* [Online]. Available: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210166Orig1s000TOC.cfm [Accessed December 17, 2020].

FDA. 2019a. *Guidance for Industry. Draft Guidance. Clinical Lactation Studies: Considerations for Study Design, May 2019* [Online]. Available: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-lactation-studies-considerations-study-design [Accessed].

FDA. 2019b. *Postapproval Pregnancy Safety Studies: (Draft) Guidance for Industry, May 2019* [Online]. Available: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/postapproval-pregnancy-safety-studies-guidance-industry [Accessed].

Gliklich, R. E., Leavy, M. B. & Dreyer, N. A., (sr eds), 2020. *Registries for Evaluating Patient Outcomes: A User's Guide, AHRQ Publication No. 19(20)- EHC020.* 4th ed. Rockville, MD: Agency for Healthcare Research and Quality.

Suares, N. C. & Ford, A. C. 2011. Prevalence of, and risk factors for, chronic idiopathic constipation in the community: systematic review and meta-analysis. *Am J Gastroenterol,* 106, 1582-91; quiz 1581, 1592.

Takeda Pharmaceuticals Company Limited. 2020. *MOTEGRITY Prescribing Information [Online]* [Online]. Available: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/210166s002lbl.pdf [Accessed] [Accessed].

Wang, J., Johnson, T., Sahin, L., Tassinari, M. S., Anderson, P. O., Baker, T. E., Bucci-Rechtweg, C., Burckart, G. J., Chambers, C. D., Hale, T. W., Johnson-Lyles, D., Nelson, R. M., Nguyen, C., Pica-Branco, D., Ren, Z., Sachs, H., Sauberan, J., Zajicek, A., Ito, S. & Yao, L. P. 2017. Evaluation of the Safety of Drugs and Biological Products Used During Lactation: Workshop Summary. *Clin Pharmacol Ther*, 101, 736-744.

Wellens, A. & Schuurkes, J. 1996. Effect of R093877 on lidamine-delayed gastric emptying of a liquid test meal in conscious dogs. *Janssen Research Foundation Preclinical Research Report.*

APPENDIX 1 SCALES AND ASSESSMENTS

The following scales/assessments will be utilized in this study:

Full Title of Scale/Assessment	Version Number	Date Issued
Ages and Stages Questionnaire	3.0	June 2009

A separate master file containing each scale/assessment listed above will be provided to the site. Updates to scales/assessments during the study (if applicable) will be documented in the table above and a new master file containing the revised scale/assessment will be provided to the site.

APPENDIX 2 ENROLLMENT/INTERIM INTERVIEW AND ADVERSE REACTIONS QUESTIONNAIRE

Enrollment

PID:_____

Demographics:

Ethnicity: □ Hispanic/ Latina □ Non-Hispanic/ Non-Latina

Race: □ Caucasian □ Black □ Asian □ Pacific Islander □ Native American/ AK Native □ Other: _____

Primary Language at Home: □ English □ Spanish □ Other _____

Education: □ <9th grade □ Junior High School (9th) □ Partial High School (10th or 11th) □ High school Graduate/ GED □ Some college (> 1 year) □ College Graduate □ Postgraduate

Total Pre-Tax Household Income: □ <$10,000 □$10,001-$49,999 □$50,000-59,999 □>$60,000 □Unknown

Current Height: _____ft _____in Current Weight: _____lbs

MOB Job title: _____ (□ Full-time □ Part-Time)

Job Description (3 job tasks): _____

Pregnancy History:

Gravidity (total # of times pregnant): _____

Parity (# of livebirths + stillbirths): _____

Spontaneous abortion (# of miscarriages/spontaneous loss): _____

Termination (# of pregnancy voluntarily terminated): _____

Breastfeeding History:

Breastfeeding experience: □ First child breastfed □ Breastfed a previous child/children

Breastfed Previous Child 1 Length of time _____ weeks/ months/ years

Breastfed Previous Child 2 Length of time_____ weeks/ months/ years

Breastfed Previous Child 3 Length of time_____ weeks/ months/ years

Breast Pump Experience:

Breast pump experience: □ First-time using a breast pump □ Previous experience with a breast pump

Familiarity with using breast pump: □ Not familiar at all □ Somewhat familiar □ Very familiar

What type of breast pump do you use for milk expression: □Electric □ Manual

Medical History:

Family Medical History: Is there anyone in your family who has had:

		Relation	Diagnosis/ Describe the condition
Birth Defect	Yes No		
Developmental Disability	Yes No		

Personal Medical History (Please note if condition is CURRENT or RESOLVED and Date Dx):

Condition	Current/ Resolved	Date Dx	Notes

Do you exercise: Yes No

If yes, how many times a week? _____

On average, how many total hours of sleep do you get in a 24-hour period? _____

Baby #1 Information:

Baby's Estimated Due Date (EDC):_____

Type of birth: □ Term (37-42) _____wks □ Pre-term (≤36) _____wks

Delivery: □ Vaginal delivery □ C-section

Birth Weight: ____lbs._____oz Birth Length: _____in or_____cm

Birth Head Circumference (OFC): _____in or_____cm

Apgar score: 1min:_____ 5 min:_____

Current Weight:_____ Current Height:_____ Current OFC:_____

Has your child been diagnosed with any abnormalities or birth defects? Or are you seeing a specialist for anything: Yes or No

If Yes, Diagnosis #1:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

If Yes, Diagnosis #2:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

Has your child been diagnosed with any serious infections or infections that required hospitalization, or any other health problems? Yes or No

If Yes, Illness #1:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

If Yes, Illness #2:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

Baby #1 Feeding Patterns:

Are you exclusively breastfeeding or do you supplement with formula (One 4-6 ounce bottle per week or more):
□ EBF □ Supplement with formula □ TPN (is in NICU)

How often and how much are they breastfed (ie: every 3 hrs, 4 ounces, etc)?

_____

_____

How do you feed your baby breast milk? Breast Only Breast and Bottle Bottle Only

How often and how much are they formula fed (ie: every 3 hrs, 4 ounces, etc)? □ Does not receive Formula

_____

_____

Does your baby eat solid foods? □ Yes □ No

At what age, did your baby start solid foods? _____ weeks/months/years

Are you currently breastfeeding any other children? □ Yes □ No (skip to page 5)

Baby #2 Information:

Baby's Estimated Due Date (EDC):_____

Type of birth: □ Term (37-42) _____wks □ Pre-term (≤36) _____wks

Delivery: □ Vaginal delivery □ C-section

Birth Weight: ____lbs._____oz Birth Length: _____in or_____cm

Birth Head Circumference (OFC):_____in or_____cm

Apgar score: 1min:_____ 5 min:_____

Current Weight:_____ Current Height:_____ Current OFC:_____

Has your child been diagnosed with any abnormalities or birth defects? Or are you seeing a specialist for anything: Yes or No

If Yes, Diagnosis #1:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

If Yes, Diagnosis #2:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

Has your child been diagnosed with any serious infections or infections that required hospitalization, or any other health problems? Yes or No

If Yes, Illness #1:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

If Yes, Illness #2:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

Baby #2 Feeding Patterns:

Are you exclusively breastfeeding or do you supplement with formula (One 4-6 ounce bottle per week or more):
□ EBF □ Supplement with formula □ TPN (is in NICU)

How often and how much are they breastfed (ie: every 3 hrs, 4 ounces, etc)?

_____

_____

How do you feed your baby breast milk? Breast Only Breast and Bottle Bottle Only

How often and how much are they formula fed (ie: every 3 hrs, 4 ounces, etc)? □ Does not receive Formula

_____

_____

Does your baby eat solid foods? □ Yes □ No

At what age, did your baby start solid foods? _____ weeks/months/years

Exposure Interview: Current medications and lifestyle habits *during the last 14 days*:

Trade/Name	Indication	Route	Dose/Unit	Freq/Unit	Dates Begin/End	Time Last Taken
Vitamins / Supplements Yes No N/A DK						
Rx meds Yes No N/A DK						
OTC meds Yes No N/A DK						
Birth control Yes No N/A DK						
Caffeine Yes No N/A DK						

Trade/Name	Indication	Route Dose/Unit Freq/Unit Dates Begin/End Time Last Taken
Alcohol Yes No N/A DK		
Cigarettes/ e-cigarettes Yes No N/A DK		
Secondhand Smoke Exposure Yes No N/A DK		
Recreational Drugs Yes No N/A DK		
Illness/ Fever Yes No N/A DK		

Have you taken any prescription medications, herbal supplements, or recreational drugs ***since birth hat you are no longer taking:*** □ Yes □ No (skip to next section)

Medication/ Herbal/ Drug Name	Dates of use

Pump and Discard:

Have you ever pumped and discarded as a result of any of the exposures you are currently taking or that you discontinued using: Yes No

If yes, for which medication/ substance and for how long did you continue to pump and discard:

Medication	Duration of Pump and Discard

Adverse Reactions:

Do you think your baby has had any of the following adverse reactions in the past 14 days?

Adverse Reaction	
Drowsiness	□ Yes □ No
Sedation or slowed breathing	□ Yes □ No
Poor feeding or refusal of the breast	□ Yes □ No
Rash	□ Yes □ No
Bruising or bleeding	□ Yes □ No
Constipation	□ Yes □ No
Diarrhea	□ Yes □ No
Stools with blood or abnormal color	□ Yes □ No
Fever	□ Yes □ No
Low Body Temperature	□ Yes □ No
Restlessness	□ Yes □ No
Irritability	□ Yes □ No
Poor Sleep	□ Yes □ No
High-pitched crying	□ Yes □ No
Abnormal movements	□ Yes □ No
Abnormal skin color	□ Yes □ No

Did you call your provider for any of these symptoms? □ Yes □ No □ Not Applicable/No symptoms

Did you visit your provider for any of these symptoms? □ Yes □ No □ Not Applicable/No symptoms

Did your provider attribute the symptoms to an exposure to a medication/substance?

□ Yes □ No □ Not Applicable/No symptoms

Have you *ever* seen any of these adverse reactions in your child since birth □ Yes □ No

If yes, which symptom(s): _____

If yes, how old was your child? _____weeks/months/years

Symptom: _____

How old was your child? _____weeks/months/years

Symptom: _____

How old was your child? _____weeks/months/years

Breast Milk Sample Information:

When is the last time you fed or pumped your baby from each breast prior to your 1st pumped study sample?

Right Breast:___:____a.m. / p.m. Left Breast:___:_____ a.m. / p.m.

Date of Collection: _____ Parent Sample ID:_____

Sample	Time	Breast Side (R or L)	Full Expression (Y or N)	Total Volume (mL)	Biospecimen ID
Sample 1 (pre-dose)					
Sample 2 (1 hour post dose)					
Sample 3 (2 hours post dose)					
Sample 4 (4hours post dose))					
Sample 5 (8 hours post dose)					
Sample 6 (12 hours post dose)					
Sample 7 (24 hours post dose)					

Collection Method: ☐ Microbiome Criteria ☐ Home Collection ☐ Mailed Milk ☐ Frozen

Treatment of Sample prior to Aliquot (No Treatment, Cooler, Refrigerator, Mailed Milk):_____

How long were the samples stored prior to Aliquot:_____

Breast Milk Sample Notes:

Interview Notes:

Interim Interview

PID:_____ Date of Interview:_____

Interviewer:_____

Breastfeeding History:

Are you still breastfeeding your child? □ Yes □ No

If No, when did you stop breastfeeding? Date:___/____/___ or Infant Age:_____weeks/ months

Infant Feeding Patterns:

Are you exclusively breastfeeding or do you supplement with formula (One 4-6 ounce bottle per week or more):
□ EBF □ Supplement with formula □ No longer breastfeeding

How often and how much are they breastfed (ie: every 3 hrs, 4 ounces, etc)?

_____

_____

How do you feed your baby breast milk? □ Breast Only □ Breast and Bottle □ Bottle Only

How often and how much are they formula fed (ie: every 3 hrs, 4 ounces, etc)? □ Does not receive Formula

_____

_____

Does your baby eat solid foods? □ Yes □ No

At what age, did your baby start solid foods? _____ weeks/months/years

Infant Health:

Has your child been diagnosed with any abnormalities or birth defects since the last interview? Or are you seeing a specialist for anything: □ Yes □ No

If Yes, Diagnosis #1:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

If Yes, Diagnosis #2:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

Has your child been diagnosed with any serious infections or infections that required hospitalization, or any other health problems since the last interview?□ Yes □ No

If Yes, Illness #1:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

If Yes, Illness #2:_____

Date Diagnosed: _____

Any Follow-up or intervention required:_____

Medication History:

Are you still taking prucalopride? □ Yes □ No

If no, when did you stop taking the medication? Date: ____/____/____

Adverse Reactions:

Do you think your baby has had any of the following adverse reactions in the past 14 days?

Adverse Reaction	
Drowsiness	□ Yes □ No
Sedation or slowed breathing	□ Yes □ No
Poor feeding or refusal of the breast	□ Yes □ No
Rash	□ Yes □ No
Bruising or bleeding	□ Yes □ No
Constipation	□ Yes □ No
Diarrhea	□ Yes □ No
Stools with blood or abnormal color	□ Yes □ No
Fever	□ Yes □ No
Low Body Temperature	□ Yes □ No
Restlessness	□ Yes □ No
Irritability	□ Yes □ No
Poor Sleep	□ Yes □ No
High-pitched crying	□ Yes □ No
Abnormal movements	□ Yes □ No
Abnormal skin color	□ Yes □ No

Did you call your provider for any of these symptoms? □ Yes □ No □ Not Applicable/No symptoms

Did you visit your provider for any of these symptoms? □ Yes □ No □ Not Applicable/No symptoms

Did your provider attribute the symptoms to an exposure to a medication/substance?

□ Yes □ No □ Not Applicable/No symptoms

APPENDIX 3 ASQ QUESTIONNAIRE

ASQ-3 Ages & Stages Questionnaires®

3 months 0 days through 4 months 30 days

4 Month Questionnaire

Please provide the following information. Use black or blue ink only and print legibly when completing this form.

Date ASQ completed: _____

Baby's information

Baby's first name: _____

Baby's date of birth: _____

If baby was born 3 or more weeks prematurely, # of weeks premature: _____

Baby's gender: ◯ Male ◯ Female

Person filling out questionnaire

First name: _____ Middle initial: _____ Last name: _____

Street address: _____

Relationship to baby: ◯ Parent ◯ Guardian ◯ Teacher ◯ Child care provider ◯ Grandparent or other relative ◯ Foster parent ◯ Other: _____

City: _____ State/ Province: _____ ZIP/ Postal code: _____

Country: _____ Home telephone number: _____ Other telephone number: _____

E-mail address: _____

Names of people assisting in questionnaire completion: _____

Program Information

Baby ID #: _____ Age at administration in months and days: _____

Program ID #: _____ If premature, adjusted age in months and days: _____

Program name: *UCSD Center for Better Beginnings - Neurodevelopmental Follow-Up Program*

ASQ-3™

4 Month Questionnaire

3 months 0 days through 4 months 30 days

On the following pages are questions about activities babies may do. Your baby may have already done some of the activities described here, and there may be some your baby has not begun doing yet. For each item, please fill in the circle that indicates whether your baby is doing the activity regularly, sometimes, or not yet.

Important Points to Remember:

- ☑ Try each activity with your baby before marking a response.
- ☑ Make completing this questionnaire a game that is fun for you and your baby.
- ☑ Make sure your baby is rested and fed.
- ☑ Please return this questionnaire by _____.

Notes:

_____

_____

_____

_____

COMMUNICATION

	YES	SOMETIMES	NOT YET	
1. Does your baby chuckle softly?	○	○	○	___
2. After you have been out of sight, does your baby smile or get excited when he sees you?	○	○	○	___
3. Does your baby stop crying when she hears a voice other than yours?	○	○	○	___
4. Does your baby make high-pitched squeals?	○	○	○	___
5. Does your baby laugh?	○	○	○	___
6. Does your baby make sounds when looking at toys or people?	○	○	○	___

COMMUNICATION TOTAL ___

GROSS MOTOR

	YES	SOMETIMES	NOT YET	
1. While your baby is on his back, does he move his head from side to side?	○	○	○	___
2. After holding her head up while on her tummy, does your baby lay her head back down on the floor, rather than let it drop or fall forward?	○	○	○	___
3. When your baby is on his tummy, does he hold his head up so that his chin is about 3 inches from the floor for at least 15 seconds? 	○	○	○	___
4. When your baby is on her tummy, does she hold her head straight up, looking around? *(She can rest on her arms while doing this.)* 	○	○	○	___

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ASQ-3 **4 Month Questionnaire** *page 3 of 5*

GROSS MOTOR *(continued)*

	YES	SOMETIMES	NOT YET	
5. When you hold him in a sitting position, does your baby hold his head steady?	○	○	○	___
6. While your baby is on her back, does your baby bring her hands together over her chest, touching her fingers? 	○	○	○	___

GROSS MOTOR TOTAL ___

FINE MOTOR

	YES	SOMETIMES	NOT YET	
1. Does your baby hold his hands open or partly open (rather than in fists, as they were when he was a newborn)? 	○	○	○	___
2. When you put a toy in her hand, does your baby wave it about, at least briefly?	○	○	○	___
3. Does your baby grab or scratch at his clothes?	○	○	○	___
4. When you put a toy in her hand, does your baby hold onto it for about 1 minute while looking at it, waving it about, or trying to chew it?	○	○	○	___
5. Does your baby grab or scratch his fingers on a surface in front of him, either while being held in a sitting position or when he is on his tummy?	○	○	○	___
6. When you hold your baby in a sitting position, does she reach for a toy on a table close by, even though her hand may not touch it?	○	○	○	___

FINE MOTOR TOTAL ___

PROBLEM SOLVING

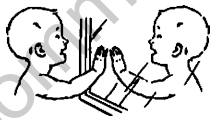
	YES	SOMETIMES	NOT YET	
1. When you move a toy slowly from side to side in front of your baby's face (about 10 inches away), does your baby follow the toy with his eyes, sometimes turning his head?	○	○	○	___
2. When you move a small toy up and down slowly in front of your baby's face (about 10 inches away), does your baby follow the toy with her eyes?	○	○	○	___
3. When you hold your baby in a sitting position, does he look at a toy (about the size of a cup or rattle) that you place on the table or floor in front of him?	○	○	○	___
4. When you put a toy in her hand, does your baby look at it?	○	○	○	___
5. When you put a toy in his hand, does your baby put the toy in his mouth?	○	○	○	___

ASQ-3 **4 Month Questionnaire** *page 4 of 5*

PROBLEM SOLVING *(continued)*

	YES	SOMETIMES	NOT YET	
6. When you dangle a toy above your baby while she is lying on her back, does your baby wave her arms toward the toy? 	○	○	○	___
			PROBLEM SOLVING TOTAL	___

PERSONAL-SOCIAL

	YES	SOMETIMES	NOT YET	
1. Does your baby watch his hands? 	○	○	○	___
2. When your baby has her hands together, does she play with her fingers?	○	○	○	___
3. When your baby sees the breast or bottle, does he seem to know he is about to be fed?	○	○	○	___
4. Does your baby help hold the bottle with both hands at once, or when nursing, does she hold the breast with her free hand?	○	○	○	___
5. Before you smile or talk to your baby, does he smile when he sees you nearby?	○	○	○	___
6. When in front of a large mirror, does your baby smile or coo at herself? 	○	○	○	___
			PERSONAL-SOCIAL TOTAL	___

OVERALL

Parents and providers may use the space below for additional comments.

1. Does your baby use both hands and both legs equally well? If no, explain: ○ YES ○ NO

2. When you help your baby stand, are his feet flat on the surface most of the time? If no, explain: ○ YES ○ NO

OVERALL *(continued)*

3. Do you have concerns that your baby is too quiet or does not make sounds like other babies? If yes, explain: ○ YES ○ NO

4. Does either parent have a family history of childhood deafness or hearing impairment? If yes, explain: ○ YES ○ NO

5. Do you have concerns about your baby's vision? If yes, explain: ○ YES ○ NO

6. Has your baby had any medical problems in the last several months? If yes, explain: ○ YES ○ NO

7. Do you have any concerns about your baby's behavior? If yes, explain: ○ YES ○ NO

8. Does anything about your baby worry you? If yes, explain: ○ YES ○ NO

ASQ-3 Ages & Stages Questionnaires®

12 Month Questionnaire

11 months 0 days through 12 months 30 days

Please provide the following information. Use black or blue ink only and print legibly when completing this form.

Date ASQ completed: _____

Baby's information

Baby's first name: _____

Baby's date of birth: _____

If baby was born 3 or more weeks prematurely, # of weeks premature: _____

Baby's gender: ◯ Male ◯ Female

Person filling out questionnaire

First name: _____ Middle initial: _____ Last name: _____

Relationship to baby: ◯ Parent ◯ Guardian ◯ Teacher ◯ Child care provider ◯ Grandparent or other relative ◯ Foster parent ◯ Other: _____

Street address: _____

City: _____ State/ Province: _____ ZIP/ Postal code: _____

Country: _____ Home telephone number: _____ Other telephone number: _____

E-mail address: _____

Names of people assisting in questionnaire completion: _____

Program Information

Baby ID #: _____ Age at administration in months and days: _____

Program ID #: _____ If premature, adjusted age in months and days: _____

Program name: _____

ASQ-3

12 Month Questionnaire

11 months 0 days through 12 months 30 days

On the following pages are questions about activities babies may do. Your baby may have already done some of the activities described here, and there may be some your baby has not begun doing yet. For each item, please fill in the circle that indicates whether your baby is doing the activity regularly, sometimes, or not yet.

Important Points to Remember:

- ☑ Try each activity with your baby before marking a response.
- ☑ Make completing this questionnaire a game that is fun for you and your baby.
- ☑ Make sure your baby is rested and fed.
- ☑ Please return this questionnaire by _____.

Notes:

COMMUNICATION

	YES	SOMETIMES	NOT YET	
1. Does your baby make two similar sounds, such as "ba-ba," "da-da," or "ga-ga"? *(The sounds do not need to mean anything.)*	○	○	○	___
2. If you ask your baby to, does he play at least one nursery game even if you don't show him the activity yourself (such as "bye-bye," "Peeka-boo," "clap your hands," "So Big")?	○	○	○	___
3. Does your baby follow one simple command, such as "Come here," "Give it to me," or "Put it back," *without* your using gestures?	○	○	○	___
4. Does your baby say three words, such as "Mama," "Dada," and "Baba"? *(A "word" is a sound or sounds your baby says consistently to mean someone or something.)*	○	○	○	___
5. When you ask, "Where is the ball (hat, shoe, etc.)?" does your baby look at the object? *(Make sure the object is present. Mark "yes" if she knows one object.)*	○	○	○	___
6. When your baby wants something, does he tell you by *pointing* to it?	○	○	○	___

COMMUNICATION TOTAL ___

GROSS MOTOR

	YES	SOMETIMES	NOT YET	
1. While holding onto furniture, does your baby bend down and pick up a toy from the floor and then return to a standing position? 	○	○	○	___
2. While holding onto furniture, does your baby lower herself with control (without falling or flopping down)?	○	○	○	___
3. Does your baby walk beside furniture while holding on with only one hand?	○	○	○	___

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ASQ-3 **12 Month Questionnaire** *page 3 of 6*

GROSS MOTOR *(continued)*

	YES	SOMETIMES	NOT YET	
4. If you hold both hands just to balance your baby, does he take several steps without tripping or falling? *(If your baby already walks alone, mark "yes" for this item.)* 	○	○	○	___
5. When you hold one *hand* just to balance your baby, does she take several steps forward? *(If your baby already walks alone, mark "yes" for this item.)* 	○	○	○	___
6. Does your baby stand up in the middle of the floor by himself and take several steps forward?	○	○	○	___

GROSS MOTOR TOTAL ___

FINE MOTOR

	YES	SOMETIMES	NOT YET	
1. After one or two tries, does your baby pick up a piece of string with his first finger and thumb? *(The string may be attached to a toy.)* 	○	○	○	___
2. Does your baby pick up a crumb or Cheerio with the *tips* of her thumb and a finger? She may rest her arm or hand on the table while doing it. 	○	○	○	___
3. Does your baby put a small toy down, without dropping it, and then take his hand off the toy?	○	○	○	___
4. Without resting her arm or hand on the table, does your baby pick up a crumb or Cheerio with the *tips* of her thumb and a finger? 	○	○	○	___*
5. Does your baby throw a small ball with a forward arm motion? *(If he simply drops the ball, mark "not yet" for this item.)* 	○	○	○	___
6. Does your baby help turn the pages of a book? *(You may lift a page for him to grasp.)*	○	○	○	___

FINE MOTOR TOTAL ___

**If Fine Motor Item 4 is marked "yes" or "sometimes," mark Fine Motor Item 2 "yes."*

ASQ-3 **12 Month Questionnaire** *page 4 of 6*

PROBLEM SOLVING

	YES	SOMETIMES	NOT YET	
1. When holding a small toy in each hand, does your baby clap the toys together (like "Pat-a-cake")?	○	○	○	___
2. Does your baby poke at or try to get a crumb or Cheerio that is inside a clear bottle (such as a plastic soda-pop bottle or baby bottle)?	○	○	○	___
3. After watching you hide a small toy under a piece of paper or cloth, does your baby find it? *(Be sure the toy is completely hidden.)*	○	○	○	___
4. If you put a small toy into a bowl or box, does your baby copy you by putting in a toy, although she may not let go of it? *(If she already lets go of the toy into a bowl or box, mark "yes" for this item.)*	○	○	○	___
5. Does your baby drop two small toys, one after the other, into a container like a bowl or box? *(You may show him how to do it.)* 	○	○	○	___ *
6. After you scribble back and forth on paper with a crayon (or a pencil or pen), does your baby copy you by scribbling? *(If she already scribbles on her own, mark "yes" for this item.)*	○	○	○	___

PROBLEM SOLVING TOTAL ___

**If Problem Solving Item 5 is marked "yes" or "sometimes," mark Problem Solving Item 4 "yes."*

PERSONAL-SOCIAL

	YES	SOMETIMES	NOT YET	
1. When you hold out your hand and ask for his toy, does your baby offer it to you even if he doesn't let go of it? *(If he already lets go of the toy into your hand, mark "yes" for this item.)*	○	○	○	___
2. When you dress your baby, does she push her arm through a sleeve once her arm is started in the hole of the sleeve?	○	○	○	___
3. When you hold out your hand and ask for his toy, does your baby let go of it into your hand?	○	○	○	___
4. When you dress your baby, does she lift her foot for her shoe, sock, or pant leg?	○	○	○	___
5. Does your baby roll or throw a ball back to you so that you can return it to him?	○	○	○	___
6. Does your baby play with a doll or stuffed animal by hugging it?	○	○	○	___

PERSONAL-SOCIAL TOTAL ___

OVERALL

Parents and providers may use the space below for additional comments.

1. Does your baby use both hands and both legs equally well? If no, explain: ○ YES ○ NO

2. Does your baby play with sounds or seem to make words? If no, explain: ○ YES ○ NO

3. When your baby is standing, are her feet flat on the surface most of the time? If no, explain: ○ YES ○ NO

4. Do you have concerns that your baby is too quiet or does not make sounds like other babies do? If yes, explain: ○ YES ○ NO

5. Does either parent have a family history of childhood deafness or hearing impairment? If yes, explain: ○ YES ○ NO

ASQ-3 **12 Month Questionnaire** *page 6 of 6*

OVERALL *(continued)*

6. Do you have concerns about your baby's vision? If yes, explain: ○ YES ○ NO

7. Has your baby had any medical problems in the last several months? If yes, explain: ○ YES ○ NO

8. Do you have any concerns about your baby's behavior? If yes, explain: ○ YES ○ NO

9. Does anything about your baby worry you? If yes, explain: ○ YES ○ NO

APPENDIX 4 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

Appendix 4.1 Regulatory and Ethical Considerations

This study is conducted in accordance with current applicable regulations including ICH E6, EU Directive 2001/20/EC, and all updates, as well as local ethical and legal requirements.

Compliance with these regulations and guidelines also constitutes compliance with the ethical principles described in the Declaration of Helsinki.

The name and address of each third-party vendor (eg, CRO [contract research organization]) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

Appendix 4.2 Sponsor's Responsibilities

Good Clinical Practice Compliance

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all applicable industry regulations, current ICH GCP guideline E6 (1996), EU Directive 2001/20/EC Guidelines, as well as all applicable national and local laws and regulations.

Visits to sites may be conducted by representatives of the study sponsor to review processes. Records and data may be reviewed at the request of regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The investigator is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to start of the study and over the course of the study until completion.

Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating investigators' names and contact information. The investigator will also post study information on appropriate websites, such as the US FDA's list of registries.

The timing for study registration and results summary posting must be in accordance with applicable local and national requirements.

Submission of Summary of Clinical Study Report to Competent Authorities of Member States Concerned and Ethics Committees

The investigator will provide the final study report to the sponsor. The sponsor will provide a summary of the study report to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the Community guideline on GCP. This requirement will be fulfilled within 1 year after the completion of the study.

Study Suspension, Termination, and Completion

The sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the sponsor will ensure that the investigator and regulatory agencies are notified. The investigator will ensure that the IRB with oversight of the study is notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

Appendix 4.3 Investigator's Responsibilities

Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6 (1996) and E6 R2 (2017), EU Directive 2001/20/EC, and applicable regulatory requirements and guidelines.

It is the investigator's responsibility to ensure that adequate time and appropriately trained resources are available prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable participants within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study-related tasks, and shall, upon request of the sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such qualification. Curriculum vitae for investigators and sub-investigators are provided to the study sponsor (or designee) before starting the study.

The principal investigator will review and agree with the final clinical study report and document this with dated signature of the principal investigator, in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

Protocol Adherence and Investigator Agreement

The investigator and any sub-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those participants who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study for any reason, the investigator will promptly inform the sponsor and the IRB/EC and provide them with a detailed written explanation. Upon study completion, the investigator will provide the sponsor, IRB/EC, and regulatory agency with final reports and summaries as required.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or for multicenter studies, the coordinating principal investigator according to national provisions and will be documented in the investigator agreement.

Documentation and Retention of Records

Paper data collection forms, questionnaires, medical records released, consent documentation and study database for the study will be retained in a secure environment at the UC San Diego investigator's site for at least 10 years after study completion.

Case Report Forms

The study report forms have been developed by the investigator and will be reviewed for comment by the sponsor.

The investigator is responsible for maintaining adequate and accurate records from which accurate information is recorded onto study report forms and database pertinent to the clinical investigation. Study report forms and database entries will be completed by the investigator or the designated study staff as stated in the site delegation log.

Summary data will be sent to the sponsor and must be endorsed by the investigator.

Recording, Access, and Retention of Source Data and Study Documents

Original source data for this study will include: participant's interview data, questionnaires, medical records released for the infant and data on sample collection and laboratory values for samples that are assayed.

All key data must be recorded in the participant's source documents.

The investigator must permit authorized representatives of regulatory authorities and the IRB of record for this study to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The IRB of record or regulatory inspectors may check the case report form (CRF) entries against the source documents. The consent form includes a statement by which the participant agrees to the regulatory authorities or the IRB having access to source data.

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (eg, the US FDA, EMA, UK Medicines and Healthcare products Regulatory Agency) or the IRB of record.

Essential documents must be maintained for at least 10 years after study completion.

Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other US national and local regulatory authorities), the EMA, the Medicines and Healthcare products Regulatory Agency, other regulatory authorities, and the IRB/EC of record for the study.

Financial Disclosure

The investigator is required to disclose any financial arrangement during the study and for 1 year after, whereby the outcome of the study could be influenced by the value of the compensation for conducting the study, or other payments the investigator received from the sponsor. The following information is collected: any significant payments from the sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in investigational product; any significant equity interest in the sponsor or subsidiaries as defined in 21 CFR 54 2(b) (1998).

Appendix 4.4 Ethical Considerations

Informed Consent

It is the responsibility of the investigator to obtain written informed consent from all study participants prior to completion of participation in the study. Initial enrollment will be completed with verbal consent administered by telephone to allow for collection of interview data. All consent documentation must be in accordance with applicable regulations and GCP. Each participant is will provide verbal agreement after the participant has read or been read the participant information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the participant's rights and responsibilities. Verbal agreement to participate will be documented by the study staff member performing the consent process prior to collection of any study interview data. Written informed consent will be provided at the time of milk collection and shipped back to the site with the milk samples. A copy of the informed consent documentation (ie, a complete set of participant information sheets and fully executed signature pages) must be given to the participant. Signed consent forms must remain in each participant's study file and must be available for verification at any time.

The principal investigator provides the sponsor with a copy of the consent form which was reviewed by the IRB and which received their favorable opinion/approval. A copy of the IRB written favorable opinion/approval of these documents must be provided to the sponsor, prior to the start of the study.

Institutional Review Board or Ethics Committee

It is the responsibility of the investigator to submit this protocol, the informed consent document (approved by the sponsor or their designee), relevant supporting information and all types of participant recruitment information to the IRB of record for review, and all must be approved prior to study start.

Prior to implementing changes in the study, the sponsor and the IRB must approve any revisions of all informed consent documents and amendments to the protocol unless there is a participant safety issue. If required by local law, substantial amendments to the protocol must also be approved by the appropriate regulatory agency prior to implementation.

The investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol, at least annually or more frequently in accordance with the

requirements, policies, and procedures established by the IRB. This is the responsibility of the investigator. The investigator must also keep the local IRB informed of any serious and significant protocol violations as required by IRB procedures.

Privacy and Confidentiality

All US-based sites and laboratories or entities providing support for this study, must, where applicable, comply with the HIPAA of 1996. The confidentiality of records that may be able to identify participants will be protected in accordance with applicable laws, regulations, and guidelines.

After participants have consented to take part in the study, the investigator obtains data collected during the study from interviews, questionnaires, medical records released by the participant and laboratory assays. These records and data may be reviewed by national or local regulatory authorities; and the IRB of record which gave approval for the study to proceed.

Personal identifying information is collected for all study participants; however, data are recorded in the study database using a unique identifying number.

Study Results / Publication Policy

The term “Publication” shall mean any paper, article, manuscript, report, poster, internet posting, presentation slides, abstract, outline, video, instructional material, presentation (in the form of a written summary), or other public disclosure of the study results, in printed, electronic, oral, or other form.

At least sixty (60) days prior to submitting an abstract, manuscript, or other document for publication, a copy of the proposed publication will be provided to the sponsor by the investigator for review. Upon the sponsor’s request, the site agrees to remove any and all confidential information (expressly excluding study results) identified in the publication and to delay such submission or presentation for an additional sixty (60) day period in order to allow the sponsor time to file any patent application(s).

Takeda is committed to transparent dissemination of all scientific, technical and medical manuscripts generated from Takeda-supported research. Therefore, after January 1, 2018, Takeda will require the submission of all Takeda-supported research manuscripts to journals that offer public availability via Open Access (including publisher platforms/repositories and self-archiving). Open Access refers to the free at point of entry, online availability of published research output with, where available, rights of re-use according to an End User License.

Unless otherwise required by the journal in which the publication appears, or the forum in which it is made, authorship will comply with the International Committee of Medical Journal Editors (ICMJE) Recommendation for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical journals.

APPENDIX 5 MANAGEMENT AND REPORTING OF ADVERSE EVENTS

All adverse events will be managed and reported in compliance with all applicable regulations and Takeda policies for reporting of adverse events (AEs) and product complaints.

Appendix 5.1 Adverse Events Definitions

An **adverse event** is any untoward medical occurrence in a subject administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with use of the product, whether or not related to the product. Worsening in severity of a pre-existing condition after administration of the product would be considered an AE.

Although abnormal laboratory values are typically not considered AEs, the following considerations may result in an abnormal laboratory value being considered an AE:

- A laboratory test result that meets the criteria for an SAE
- A laboratory test result that requires the subject/patient to receive specific corrective therapy
- A laboratory abnormality that leads to discontinuation of therapy
- A laboratory abnormality that the health care provider considers to be clinically significant

A **serious adverse event** is any untoward medical occurrence that at any dose:

- Results in death. Note that death is an outcome of an event. The event(s) causing death should be recorded; or
- In the view of the health care provider, places the subject/patient at immediate risk of death (a life threatening event); however, this does not include an event that, had it occurred in a more severe form, might have caused death; or
- Requires inpatient hospitalization or prolongation of existing hospitalization or;
- Results in persistent or significant disability/incapacity; or

An SAE may also be any other medically important event that, in the opinion of the health care provider, may jeopardize the subject/patient or may require intervention to prevent one of the other outcomes listed in the definition above.

A **product quality issue** (PQI) refers to defects related to the safety, identity, strength, quality, or purity of the product or with the physical characteristics, packaging, labeling, or design of the product.

A **special situation report** (SSR) includes any of the following events:

- Overdose: All information of any accidental or intentional overdose

- Drug abuse, misuse or medication error: All information on medicinal product abuse, misuse or medication error (potential or actual)
- Suspected transmission of an infectious agent: Suspected (in the sense of confirmed or potential) transmission of an infectious agent by a medicinal product.
- Lack of efficacy of Takeda Product
- Accidental/Occupational exposure
- Use outside the terms of the marketing authorization, also known as “off-label”
- Use of falsified medicinal product
- Use of counterfeit medicinal product
- Drug-drug interactions and drug-food interactions
- Inadvertent or accidental exposure with or without an AE
- Unintended benefit
- Pregnancy: Any case in which a pregnancy patient is exposed to a Takeda Product or in which a female patient or female partner of a male patient becomes pregnant following treatment with Takeda Product. Exposure is considered either through maternal exposure or via semen following paternal exposure.
- Breastfeeding: Infant exposure from breast milk

An SSR shall be reported even if there is no associated AE if attributed to prucalopride exposure in accordance with reporting procedures described in Appendix 5.2 and Appendix 5.2.1.

Relationship Categorization: The process for relationship categorization is further described in Appendix 5.2.4.

Assigning a relationship of an AE to prucalopride is based on the consideration of all available information about the event, including the temporal relationship to drug administration, recognized association with drug product/class, pharmacological plausibility, and alternative etiology (e.g., underlying illness, concurrent conditions, concomitant treatments).

- *Related (Yes):* An AE that follows a reasonable temporal sequence from administration of the prucalopride (including the course after withdrawal of the medication), and for which a causal relationship is compelling enough and/or follows a known or suspected response pattern and the event cannot be explained by the subject’s other underlying diseases, complications, concomitant drugs and concurrent treatments.

- *Not related (No):* An AE that does not follow a reasonable temporal sequence from administration of the prucalopride and/or that can reasonably be explained by other factors, such as the subject’s underlying disease, complications, concomitant drugs and concurrent treatments.

Identifiable safety information includes any SAE, AE, SSR, or PQI where the Takeda product is known and at least one demographic is known for reporter and the patient.

Takeda awareness date is the date when any person working on behalf of Takeda whether as an employee, consultant, contractor, or in any other capacity becomes aware of a safety information irrespective of whether the information becomes known during a weekend or public holiday.

Medical and scientific judgment shall be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

Appendix 5.2 Collection of Adverse Events

Serious AEs (Appendix 5.1) that are not systematically collected per the study protocol may be reported as spontaneous reports (European Medicines Agency (EMA) guideline on good pharmacovigilance practices (GVP) module VI, section VI.B.1.2. (EMA, 2017).

The following reportable events will be collected by the Human Milk Biorepository (HMB) Research Center and reported to Takeda within 1 business day of becoming aware of the event on a MedWatch form:

- Serious AEs that are systematically collected per protocol (Appendix 5.2.1) involving:
    - Hospitalization in infants up to 1 year of age
    - Hospitalization of the mother
    - Death of infant
    - Death of mother
- Any event (AE, SAE, SSR, and PQI) that is specifically attributed to prucalopride by the reporter, regardless of seriousness

The HMB Research Center may be contacted by Takeda to obtain additional information on the event or for data clarification. The HMB Research Center shall make their best effort to obtain the requested additional information and will notify Takeda within 1 working day of obtaining the additional information for a fatal or life-threatening SAE, within 4 calendar days for other SAEs, and within 7 calendar days for all other events/issues.

Appendix 5.2.1. Targeted AEs

At each study interview, participants who continue to breastfeed their infant will be asked to respond to a predefined checklist to solicit the following AEs occurring in their infant over the previous 14 days:

- Drowsiness
- Sedation or slowed breathing

- Poor feeding or refusal of the breast
- Rash
- Bruising or bleeding
- Constipation
- Diarrhea
- High-pitched crying
- Abnormal movements
- Abnormal skin color

An open-ended AE question will also be asked at each study interview to record any event that was not reported on the predefined checklist. For each reported event, the participant will be asked if the infant was hospitalized or died as a result of the event.

These events will be collected on the Adverse Reactions questionnaire to address the study objectives (Appendix 2).

The HMB Research Center will report these targeted events to Takeda quarterly unless a mother said the baby was hospitalized or died, at which time, the event would be considered serious and reported within the 1 business day timeline specified in Appendix 5.3.1. These events will also be summarized in the interim and final reports.

Appendix 5.2.2. Adverse Event Information not Collected

The European Medicines Agency (EMA) guideline on good pharmacovigilance practices (GVP) module VI section VI.C.1.2.1.1. accommodates for specific protocol-defined events for which reporting is not required (EMA, 2017). *Breastfeeding* (defined as a SSR in Appendix 5.1) will not be reported because it is an eligibility requirement in the study population. Pregnancy will be reported if it meets the criteria described in Appendix 5.2.

Appendix 5.2.3. Non-serious Events

Study personnel have frequent contact with the mother for data collection. During the course of data collection, non-serious events (solicited or unsolicited) that are common and expected during infancy may be communicated to study staff. Unsolicited non-serious events (unless attributed to prucalopride by the reporter) will not be collected.

The justification for not collecting these non-serious events is, as follows:

Outside the scope of the study. This study protocol was developed primarily to fulfill a postmarketing requirement with the overall goal of identifying an unexpected serious risk associated with the presence of prucalopride, or its active metabolite, in human breast milk. (FDA, 2018). Specifically, the study is designed as a milk only lactation trial in lactating women who have received therapeutic doses of Motegrity (prucalopride) using a validated assay to assess concentrations of prucalopride in breast milk and the effects on the breastfed infant. Protocol-specified events will be systematically collected and presented descriptively, as there is

no comparator group in this study, in the interim and final analyses. Safety data obtained from this study will supplement information received from analyses of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA (FDA, 2018).

Reporter burden. Additional data collection efforts required to characterize non-serious events may increase reporter burden. Streamlining data collection processes and establishing and maintaining a longitudinal relationship between participant and interviewer are recommended to improve participant retention and minimize losses to follow-up (FDA, 2019b). Reducing reporter burden is particularly important for studies in which the mother is the primary reporter who may be balancing work and childcare responsibilities (FDA, 2019b).

Other reporting avenues. The mother, associated healthcare professionals, and caregivers shall be informed of the possibility to report directly to the relevant Takeda Pharmacovigilance department or national pharmacovigilance reporting system any adverse events not being collected as part of the study. These will be treated as spontaneous reports and independent of the study.

Appendix 5.2.4. Relationship to Prucalopride

Assessment of causality. Upon receipt of events submitted to Takeda by the site staff, Takeda will assess relationship based on available information. The HMB Research Center may be contacted by Takeda to obtain additional information on the event or for data clarification.

The justification for not conducting a relationship assessment at the site is as follows:

A lack of evidence on the availability of prucalopride in human breast milk to evaluate exposure and relationship. For most medications, lactating women are actively excluded from clinical trials. Consequently, at the time of the initial marketing, there may be no human toxicity data to inform the availability and safety of a medication in breast milk (Wang et al., 2017). Therefore, it is the goal of this milk-only lactation study is to assess concentrations of prucalopride in breast milk and the effects on the breastfed infant (FDA, 2018; FDA, 2019b). If the concentration of a drug in breast milk is found to be clinically relevant, this finding could lead to further studies (FDA, 2019a)

No direct interaction with mother or infant participant. In this call center-based study, maternal interview(s), medical record reviews, and maternally-completed developmental screening questionnaires are the data sources, in addition to the drug concentration laboratory results.. Other than telephone interviews, study personnel have no direct contact with the mother or infant, do not provide any type of healthcare, and have limited information on medical history and comorbidities. Thus, the study has inadequate information to assign relationship to prucalopride. Adverse events collected in this study will be reported in aggregate in the interim and final study reports, which are submitted to the FDA annually (Gliklich et al., 2020).

No direct contact with healthcare providers. Healthcare providers may refer patients for this study; however, in all cases the mother is the individual who provides informed consent for participation and completes the interview-based data collection. Health care providers are only contacted when asked to submit copies of medical records.

Appendix 5.3 Reporting of AEs to Regulatory Agencies and IRB/EC

Appendix 5.3.1. Obligations of the HMB Research Center

During the course of the study, the HMB Research Center will communicate all reportable safety information (as defined in Appendix 5.2) to Takeda within one business day (but not to exceed 3 calendar days if received before a weekend or a holiday) of the awareness date to the appropriate contact (Appendix 5.3.2). The regulatory clock for reporting of safety information to Takeda begins when any person associated with this study becomes aware of identifiable safety information. The HMB Research Center submits these events on a MedWatch form to Takeda for subsequent reporting to the FDA by Takeda, as applicable.

Takeda is responsible for subsequently reporting serious and non-serious events suspected of being related to Takeda products to regulatory authorities. HMB is responsible for reporting adverse drug reactions to the IRB, if required by national law or regulation, within the timelines required by such law or regulation.

The investigator shall maintain records of all such submissions. A listing of all reported events will be maintained by HMB and reviewed quarterly with the Sponsor throughout and at the end of the study for review and reconciliation.

Appendix 5.3.2. Reporting Contacts

Adverse events reports and SSRs shall be sent to the following contacts

- Phone : [redacted]
- Email : [redacted]
- Fax : [redacted]

Product and Quality Complaints shall be sent to:

- Phone: [redacted] (US-only)
- Email: [redacted]