Title	**Observational Study to Assess Maternal and Fetal Outcomes Following Exposure to Prucalopride during Pregnancy**
Version identifier	3.0
Date of last version	April 09, 2020
EU PAS Register No:	N/A
Protocol Number	TAK-555-5002
Active substance	prucalopride
Medicinal product(s):	Motegrity
Product reference:	
Procedure number:	*N/A*
Marketing authorisation holder(s)	Shire Development LLC
Joint PASS	N/A
Research question and objectives	To assess the risk of major congenital malformations, preterm delivery, small for gestational age, and NICU admission associated with Prucalopride use in pregnancy.
Country(-ies) of study	United States
Author(s)	[redacted], MD DrPH (Harvard T.H. Chan School of Public Health) [redacted], MD MS (Brigham and Women's Hospital, Harvard Medical School) [redacted], MS PhD (Brigham and Women's Hospital, Harvard Medical School)
MAH contact person	[redacted] Global Regulatory Affairs Takeda Pharmaceutical Company Limited 350 Massachusetts Avenue Cambridge, MA 02142

SIGNATURE PAGE

Motegrity Pregnancy Exposure Retrospective Cohort Study
Protocol: TAK-555-5002

Sponsor:
[redacted], MS
[redacted]
Global Outcomes Research and Epidemiology
Takeda Pharmaceutical Company Limited
55 Hayden Ave, Lexington, MA 02421

Signature and Date: [redacted]

Investigator:
[redacted], MD DrPH
Harvard T.H. Chan School of Public Health
677 Huntington Ave, Boston, MA 02115

Signature and Date: _____

SIGNATURE PAGE

Motegrity Pregnancy Exposure Retrospective Cohort Study
Protocol: TAK-555-5002

Sponsor:
[redacted], MS
[redacted]
Global Outcomes Research and Epidemiology
Takeda Pharmaceutical Company Limited
55 Hayden Ave, Lexington, MA 02421

Signature and Date: _____

Investigator:
[redacted], MD DrPH
Harvard T.H. Chan School of Public Health
677 Huntington Ave, Boston, MA 02115

Signature and Date: [redacted]

TABLE OF CONTENTS

SIGNATURE PAGE 2
TABLE OF CONTENTS....3
LIST OF TABLES....5
LIST OF ABBREVIATIONS....6
1. SUMMARY....7
2. BACKGROUND....9
2.1 Treatment of Constipation....9
2.2 Treatment of Constipation During Pregnancy....9
2.3 Gaps in Knowledge for Prucalopride....10
2.4 Rationale for a Health Care Utilization Database Study....10
2.5 Rationale for choosing IBM Health MarketScan....11
3. OBJECTIVES....13
4. RESEARCH METHODOLOGY....14
4.1 Study Design....14
4.2 Data Source....18
4.3 Source Population....18
4.4 Study Population....19
4.4.1 Inclusion Criteria....19
4.4.2 Exclusion Criteria....20
4.5 Exposure Definitions....20
4.5.1 Exposure Ascertainment....20
4.5.2 Exposure and Reference Groups....21
4.5.3 Timing of Exposure:....21
4.6 Outcome definitions....23
4.6.1 Spontaneous Abortions and Stillbirths....23
4.6.2 Major Congenital Malformations....23
4.6.3 Preterm Delivery, Small for Gestational Age and NICU Admission....25
4.7 Patient Characteristics....25
4.8 Data analysis....27
4.8.1 Primary Analysis....27
4.8.2 Secondary Analyses....29
4.8.3 Interim Monitoring....31
4.9 Strengths of the Study Design....31
4.9.1 Large, Population-based Cohort....31
4.9.2 Objective Assessment of Drug Exposure....31
4.9.3 Detailed Information of Potential Confounders....31

4.10 Limitations of the Research Methods....31
4.10.1 Exposure Misclassification....31
4.10.2 Outcome Misclassification....32
4.10.3 Comparator Groups....32
4.10.4 Residual Confounding....33
4.10.5 Selection Bias....33
4.10.6 Other Missing Information....33
4.10.7 Statistical Power....34
4.11 Study Size....34
5. PROTECTION OF HUMAN SUBJECTS....36
5.1 Ethical Approval and Subject Consent....36
5.2 Subject Confidentiality....36
6. MANAGEMENT AND REPORTING OF ADVERSE EVENTS....37
7. REFERENCES....38

LIST OF TABLES

Table 4.1 Summary of Study Design Including Insurance Eligibility Requirements for Mothers and Offspring, Prucalopride Exposure Windows, Outcome Assessment Windows, and Covariate Assessment Windows 17
Table 4.2 Covariates Included in Each of the Analyses and the Associated Covariate Assessment Window 27
Table 4.3 Pre-specified Sensitivity Analyses 30
Table 4.4 Power to Detect Associations Based on the Number Exposed and Relative Risks 35

LIST OF ABBREVIATIONS

AE	Adverse Event
ADR	Adverse drug reaction
BMI	Body mass index
CDC	Centers for Disease Control
CI	Confidence interval
CPT	Current Procedure Terminology
ID	Identifier
IRB	Institutional Review Board
LMP	Last menstrual period
MAH	Marketing Authorization Holder
MAX	Medicaid Analytic eXtract
NBDPS	National Birth Defects Prevention Study
NDC	National Drug Codes
NICU	Neonatal Intensive Care Unit
OTC	Over the counter
PS	Propensity score
PPV	Positive Predictive Value
QC	Quality Control
RR	Relative Risk
SAE	Serious adverse event
SAR	Serious adverse reaction
SD	Standard Deviation
SGA	Small for gestational age
US FDA	United States Food and Drug Administration

1. SUMMARY

Rationale and background: There are no published controlled studies examining the safety of prucalopride in pregnancy. Patients and prescribers need information on the safety of prucalopride in pregnancy to decide whether the benefits outweigh the risks.

Research question and objectives: To determine whether exposure to prucalopride during pregnancy increases the risk of pre-specified major adverse maternal and fetal outcomes.

Study design: Retrospective observational cohort study with prospective data entry.

Population: Privately insured US pregnant women 16 to 44 years of age with a clinical diagnosis of constipation.

Exposure: Women who filled at least one prescription for prucalopride during the etiologically relevant time window (which varies depending on the outcome studied) will be compared to women with constipation not exposed to prucalopride and with (primary reference) or without (secondary reference) prescriptions for other drugs with similar indication. Maternal exposure to prucalopride and active comparators will be determined based on pharmacy dispensing records.

Outcomes: Outcomes will be defined based on the presence of inpatient and/or outpatient diagnoses and procedures, using validated definitions. The primary outcome measure is major congenital malformations, other outcomes are considered secondary. In the overall pregnancy cohort, outcomes include spontaneous abortions and stillbirths. In the cohort of pregnancies ending in a live-birth, outcomes include major congenital malformations (overall), preterm birth, small for gestational age, and admission to neonatal intensive care unit (NICU). Organ-specific malformations will be described but numbers are expected to be too small for formal analysis.

Covariates: Covariates that could potentially confound the association between prucalopride exposure and the outcomes of interest include medical indications for prucalopride (constipation severity), maternal demographic characteristics, comorbid medical conditions, obstetric characteristics/conditions, other maternal medications, and measures of healthcare utilization.

Data sources: IBM Health MarketScan, a large population-based claims database that includes privately insured women, for the period (estimated) 2019-2022. These years of data will be available from MarketScan in 2021-2024. The study period will depend on utilization and would be expanded as data become available to up to end of study. If target number of exposed pregnancies is not met, we will consider expanding the years of MarketScan data, adding another datasource, or terminating the study.

Study size: Prucalopride was approved and indicated for treatment of chronic idiopathic constipation (CIC) in adults in the US in December 2018 and became commercially available as of April 2019. The study will include all women exposed during pregnancy as data accumulate. It is difficult to project utilization and sample size and therefore monitoring of number of exposed as data accumulate by 2022 and 23 will inform more realistic sample size estimates. Meanwhile, preliminary analyses will allow the creation of the cohort of pregnancies diagnosed with constipation, development of algorithms for covariates, characterization of the population

and overall patterns of constipation diagnoses and prescriptions, and preparation of more detailed statistical protocols for prucalopride.

Data analysis: Results will be presented for three levels of adjustment: (i) crude analysis in the population restricted to women with recorded constipation or laxative dispensings during pregnancy, (ii) restricted to women with recorded constipation or laxative dispensings, using propensity score (PS) stratification to further control for proxies of constipation severity, concomitant medications (e.g. antidepressants), comorbidities (e.g. diabetes) and other potential confounders, and (iii) restricted and PS stratified analysis using an active comparator group (standard constipation prescription drug) to further reduce residual confounding. Generalized linear models will be used to estimate relative risks and their corresponding 95% confidence intervals comparing the exposed group with the appropriate reference group. Sensitivity analyses will be conducted to test the robustness of the findings. For example, by evaluating associations with two or more dispensing during the window of interest as a proxy for cumulative dose and adherence.

Milestones: Milestones include two phases with a gap of 2-3 years in between as utilization permeates and data on exposed pregnancies accumulates:

- Phase 1 (2019) 1) Development of protocol, and 2) review of protocol with sponsor
- Phase 2 (approx. 2022-2024): 1) data management, including data purchase, programming, data management, data use agreements and IRB approvals, 2) monitoring of utilization and development of detailed statistical protocol, 3) data analysis and interpretation, 4) report (s) to sponsor, 5) scientific manuscript, 6) public presentation of results at scientific conference (s). Analyses are expected to start by September 1, 2022 (assuming a minimum of 100 exposed pregnancies by 2022 in the database; i.e. pregnancies exposed before 2020) and would be completed by September 30, 2024.

The start of data collection is anticipated to occur after drug was commercially available, when the first date prucalopride is dispensed (anticipated Q2 2019). This date depends on drug uptake, local reimbursement, administrative procedures within MarketScan, and drug uptake in the population included in the study (women of childbearing age). The study will start when data from both 2019 and 2020 is available from MarketScan (anticipated Q1 2022) and data extraction will end after approximately three years of patient accrual (anticipated Q2 2024). The number of prucalopride-exposed pregnancies will be monitored every year to inform the study size, update the predicted study power, and inform the statistical analysis. Monitoring of drug uptake is planned to start in Q2 2022. The end of the data collection for secondary data use, defined as the date from which the analytical data set is available for the analysis, is planned for Q2 2024. The statistical analysis plan will be developed before the data for the main analysis are extracted and will provide details on the study core analyses and sensitivity analysis. Analyses and final report would be completed during Q3-Q4 2024.

Interim Reports: Interim progress reports will be submitted yearly between Q3 2021 and Q3 2024. Prucalopride use counts reports are planned to start in Q3 2022, reports with comparisons in Q4 2024 and the final study report is planned for Q3 2025. Study timelines will be confirmed later based on drug monitoring.

2. BACKGROUND

2.1 Treatment of Constipation

Chronic constipation is highly prevalent in adults. When diet (i.e., drinking enough fluids, eating more fiber) and exercise are not enough to improve bowel movements, laxatives, such as a bulk forming agent (e.g., Metamucil), osmotic agent (e.g., Miralax), stool softener (e.g., Colace), or lubricant type are recommended. Stimulant laxatives (e.g., Correctol, Dulcolax) are generally reserved for when other types are not effective.[1] New classes of laxatives for chronic idiopathic constipation approved in the last decade include lubiprostone, a chloride channels activator that increases fluid in the digestive tract; linaclotide and plecanatide, guanylate cyclase-C agonists that make bowel movements more regular; and prucalopride, a prokinetic, high affinity 5-HT4 receptor agonist that normalizes bowel movements.[2]

2.2 Treatment of Constipation During Pregnancy

Constipation affects approximately a third of all women at some point during their pregnancy.[3, 4] The higher frequency during pregnancy is presumably due to hormones that relax the intestinal muscle and by the pressure of the expanding uterus on the intestines.[5] Not surprisingly, stool softeners are among the most commonly used drugs during pregnancy, including the first trimester.[6, 7] Docusate (e.g., Colace) has been the most common drug used to treat or prevent constipation during pregnancy in the US. What is striking about these gastrointestinal drugs is the absence of information on safety in pregnant populations. There are no controlled studies in humans. Although no excess risk of major birth defects were reported by the Collaborative Perinatal Project, the analysis included only 30 in-utero exposures.[8] Therefore, there is insufficient evidence to assess the safety of constipation treatments in pregnancy.[9] However, gentle laxatives (bulking, osmotic, softeners) are generally considered safe, mainly because of their limited systemic absorption;[10] while stimulant laxatives are avoided because they might stimulate uterine contractions. There is a dearth of information available for use of newer prescription laxatives and they are thus not recommended during pregnancy. Any laxative can induce diarrhea, leading to fluid loss, electrolyte disturbances, and dehydration; adverse effects that are particularly concerning in pregnant women that may negatively impact on pregnancy outcomes.[11, 12]

For pregnant women, risk factors for constipation include lack of exercise, insufficient fluids, low fiber diets, as well as certain chronic conditions and medications.[13] Diseases associated with constipation include irritable bowel syndrome, hypothyroidism, diabetes, celiac disease, non-celiac gluten sensitivity, and inflammatory bowel disease. Some of these conditions are relatively prevalent in pregnancy (e.g. diabetes) and might be associated with adverse pregnancy outcomes. Medications that have constipation as a side effect include opioids (users excluded), diuretics, antidepressants, antihistamines, antispasmodics, anticonvulsants, tricyclic antidepressants, antiarrythmics, beta-adrenoceptor antagonists, calcium channel blockers, anticholinergics, anti-diarrheals, 5-HT3 receptor antagonists such as ondansetron, aluminum antacids, and calcium and iron supplements.[1]

2.3 Gaps in Knowledge for Prucalopride

Prucalopride was approved for use in Europe in 2009 but has only been recently approved by the US Food and Drug Administration (FDA), on 14 December 2018, for the treatment of chronic idiopathic constipation (CIC).[14, 15] Prucalopride is a selective, high affinity 5-hydroxytryptamine type 4 (5-HT4) receptor agonist with enterokinetic properties. By stimulating the serotonin 5-HT4 receptor in the colon it enhances colonic peristalsis, which provides the propulsive force for defecation. This is a different mechanism of action than that of osmotic laxatives and prosecretory products.

There are no published controlled studies examining the safety of prucalopride in pregnancy. However, a pharmacoepidemiology study consisting of 3 substudies was conducted using data drawn from the Clinical Practice Research Dataset (CPRD) in the United Kingdom. One substudy was designed as a pregnancy surveillance analysis designed to characterize pregnancy outcomes in a cohort of women exposed to prucalopride during their pregnancy. No comparator group was included as part of the pregnancy surveillance substudy. Pregnancy exposure to prucalopride was defined as any prucalopride prescription within 45 days prior to the first pregnancy-specific record or during pregnancy. Pregnancy outcomes were classified as live birth, elective pregnancy termination, spontaneous abortion or still birth, and presence and type of major malformations determined based on Read codes and a general practitioner questionnaire. In this substudy, 14 pregnancies in 12 women were classified as exposed to prucalopride, with all exposures occurring during the first trimester. Pregnancy outcomes consisted of 5 live births, 4 spontaneous abortions, three elective pregnancy terminations, and two indeterminate outcomes. No fetal malformations were identified.

2.4 Rationale for a Health Care Utilization Database Study

Generally, both the efficacy and the most common adverse effects of medications in adults and children are identified in clinical trials conducted before a given drug is approved for marketing. When it comes to pregnancy safety, however, the situation is reversed: Since pregnant women are excluded from most clinical trials, we learn about most maternal and fetal toxicities only after a drug has been marketed, and of course, only after it has been used by pregnant women. In the post-marketing setting, health care utilization databases such as the IBM Health MarketScan Database have become a standard source of information. They provide prospectively collected information for large populations and allow the study of multiple outcomes. The large size of these datasets often generates enough statistical power to examine some rare outcomes and important subgroups. While studies emerging from these databases lack the benefits of randomization, if carefully designed the results have been shown to be valid and informative, particularly when evaluating unintended drug effects.[16]

The evaluation of constipation drugs overall using administrative databases would be challenging given that many of them are available over the counter (OTC) and therefore purchased without a prescription. However, this limitation does not affect prucalopride since it is currently a prescription medication. Another limitation of real-world data is the lack of treatment randomization. That is, women with severe chronic constipation that requires prescription medications are not comparable to unexposed women in the general population. To reduce

potential confounding we propose to restrict the study population to women with the indication of constipation (much like a hypothetical clinical trial with indication as an inclusion criterion). Moreover, women on prucalopride cannot be compared to women with untreated constipation, or even on gentle OTC laxatives, since these women may have a less severe disease. For this reason, we propose to use as an active reference group those women on other prescription drugs indicated for constipation (e.g., linaclotide, lactulose, lubiprostone, plecanatide). Although health care providers can also prescribe OTC drugs (e.g. lactulose, docusate sodium, bisacodyl, senna, glycerol suppositories) and these drugs will also be considered. The analysis would further adjust for specific indications and other clinical and sociodemographic characteristics. Specifically, prescription constipation medications are typically recommended for people with chronic constipation or irritable bowel syndrome with constipation. Some are also recommended for people with opioid-induced constipation.

Regarding the selection of outcomes, since prucalopride might affect the smooth uterine muscle, we will focus on common pregnancy outcomes related to placentation and uterine contractions including spontaneous abortions, stillbirths, preterm birth, small for gestational age, and admission to neonatal intensive care unit (NICU). In addition, a main concern for any new drug is the potential for teratogenicity. Therefore, we will include major congenital malformations as our primary outcome of interest.

In summary, we propose to identify a cohort of pregnancies with constipation chronic enough as to receive prescription medications nested within the IBM Health MarketScan Database to provide information on the safety of prucalopride during pregnancy relative to alternative drugs.

2.5 Rationale for choosing IBM Health MarketScan

The specific data source was determined in a feasibility evaluation conducted to identify the most appropriate sources for this study. We considered very large health care databases in the United States with availability of mother-infant linked data, population-based sample, large number of pregnancies and demonstrated ability to generate valid evidence on the safety of drugs during pregnancy. Potential candidates included:

- The Medicaid Analytic eXtract (MAX). This cohort has been used extensively for studies of the safety of medications in pregnancy. The strengths of this datasource for studying the safety of prucalopride include the following: (1) The MAX is a large, population-based cohort. (2) It allows for objective prospective assessment of drug exposure through filled prescriptions. In contrast to many studies of drug exposures during pregnancy, this approach is not subject to recall bias. (3) It contains detailed information regarding conditions that can potentially confound the association between drug exposures and pregnancy outcomes. (4) It represents a diverse vulnerable population of women enrolled in Medicaid. (5) It allows partial validation of diagnoses with medical records. (6) The epidemiologists at the Harvard Pregnancy Team that would conduct the study in MAX have a decade of experience conducting pregnancy safety studies in health care databases and have validated algorithms to identify the pregnancy outcomes of interest. However, the use of new drugs is expected to be delayed in Medicaid, and there is a four years gap between data recording and availability of the database for research (data from 2015 has just been released in Q4 2019).

- HealthCore Integrated Research Database and Optum Dynamic Assessment of Pregnancies and Infants data. These are commercial databases that also allow longitudinal prospective assessment of drugs, confounders and outcomes; and include real world evidence (i.e., not restricted to volunteers). The uptake of prucalopride in commercially insured individuals is expected to be faster than in the Medicaid population. Optum has the ability to access medical records for data abstraction and validation of study endpoints; and claims data in Healthcore can be linked to medical records. It is important to clarify that the access to medical records does not mean ability to validate precisely the cases (e.g., malformations) from the exposed or reference group. Typically, less than 50% of all records can be retrieved.[17] Moreover, inclusion of an attempt to validate these specific cases would delay the production of evidence for at least one year, since it can only be done after the cohort is identified and the cases ascertained in the database; and then investigators need to obtain approval, retrieve records from multiple hospitals, and adjudicate the cases for the subsample obtained. Regarding linkage with birth certificates, which has been used to support partial outcome validation for malformations, studies have shown that birth certificates can be inaccurate records of birth anomalies and are not recommended as gold standards.[18]

- IBM MarketScan. This database represents claims information from one of the largest commercially insured populations in the US; it contains the largest number of pregnancies among the three commercial databases considered. The Harvard Pregnancy Team has experience with this datasource and has already completed large-scale validation studies involving chart review designed to optimize the accuracy of algorithms for determining the last menstrual period and identifying obstetric and fetal outcomes. MarketScan does not have access to medical records. However, definitions for and algorithms to identify study endpoints will be identical to those developed by this group based on MAX. The positive predictive values are expected to be applicable to MarketScan because the claims are coded at the level of clinical care (clinical visits and hospital discharges) before being sent to the different healthcare plans.

Overall, prioritizing the number of anticipated exposed pregnancies and the track record of the investigators conducting the study, we propose IBM MarketScan as the datasource. However, if target numbers are not met by 2024, we will consider the addition of other databases. Counts of exposed in 2022 and 2023 will inform the projections.

3. OBJECTIVES

The objective of this study is to assess the safety of prucalopride in pregnant women with constipation. Specifically, to determine whether exposure to prucalopride during specific gestational periods is associated with an increased risk of pre-specified obstetric and neonatal outcomes, we will quantify the risk in pregnancies exposed to prucalopride relative to women treated with standard prescription therapy within a population-based cohort of commercially insured pregnant women in the US with clinically diagnosed constipation.

The primary study aim is:

- To assess the relative risk of major congenital malformations overall in relation to first trimester exposure to prucalopride. Organ-specific malformations will be considered a secondary outcome since numbers are expected to be too small for formal analysis.

The secondary study aims are:

- To assess the relative risk of spontaneous abortions and stillbirths in relation to prucalopride exposure during the three months preceding the diagnosis of pregnancy loss.
- To assess the relative risk of preterm birth, growth restriction (infants born small for their gestational age) and admission to neonatal intensive care unit (NICU) in relation to early and late pregnancy exposure to prucalopride.

4. RESEARCH METHODOLOGY

4.1 Study Design

We will conduct a cohort study nested in the nationwide IBM MarketScan Database for the period April 2019 onwards to include the most recent data available. The April 2019 date reflects the month and year when prucalopride became available in the US.

We will identify pregnancies resulting in pregnancy losses or livebirths in women 16 to 44 years of age. Completed pregnancies with a live born will be linked to liveborn infants. We have developed a linkage algorithm based on state, insurance case number (which identifies family units), and date of delivery which has been used to accurately link mother-infant data files.[19] Several steps of data cleaning are implemented to ensure accurate linkage and to avoid duplication of pregnancies. Strict eligibility criteria are then applied to ensure complete claim information for the mother and infant. The efficiency of linkage of delivery admissions to infants was 74%. The study cohort will include only women with a clinical diagnosis of constipation (i.e., with the indication). To ensure complete capture of exposure, outcomes, and covariates recorded in the claims, we will impose requirements for Health Insurance eligibility that will be applied to both the mother and the offspring. These vary based on the outcome being considered and are delineated in Table 4.1. Generally, women are required to have insurance coverage with full prescriptions benefits at least during the period from 3 months before the last menstrual period (LMP) through one month after end of pregnancy. Infants are required to have insurance coverage for at least the first month after birth unless they died sooner.

We will consider the following exposure groups: prucalopride versus only non-prucalopride constipation drugs. If sample size allows we will divide prucalopride use into prucalopride with or without other constipation medications. Maternal use of prucalopride and other medications will be determined based on pharmacy dispensing records. Exposure will be defined based on a dispensed prescription for prucalopride during the etiologically relevant window for the outcome of interest.

In descriptive analyses, the frequency of the selected pregnancy outcomes will be reported for women without constipation and compared with women with constipation (with and without treatment). This comparison will allow us to assess whether constipation, or characteristics associated with its occurrence or recording, is associated with adverse pregnancy outcomes. However, to evaluate associations, the cohort will be restricted to women with constipation and the primary reference group will consist of women exposed to prescription constipation drugs other than prucalopride during the corresponding relevant window (which varies for each outcome). The secondary reference group will include women with a clinical diagnosis of constipation without prucalopride prescriptions but with or without prescriptions for other constipation therapy. Comparisons to women exposed to other drugs for the same indication address questions about their comparative safety. Active comparator references both respond to the clinically relevant question (i.e., which medication is safer) and are superior for confounding control. However, if the intention is to assess any absolute effect it can be challenging to identify a relevant active comparator that is widely used and for which definitive safety data are available. Another disadvantage of these active reference approach is the reduced power because

of the usually smaller size of the reference group. For these reasons we propose to also include comparisons to unexposed women with the same indication and thus address questions about the safety of a drug versus no pharmacological treatment but same indication. The disadvantage of this approach is the potential residual confounding by indication, if severity of the underlying disease is strongly associated with the outcome of interest.

Outcomes will be defined based on the presence of inpatient and/or outpatient diagnoses and procedures. The primary study outcome will be major malformations overall. The secondary study outcomes will include: spontaneous abortions, stillbirths, preterm delivery, small for gestational age (SGA) and NICU hospital admission. Outcomes including spontaneous abortions and stillbirths will be evaluated among all pregnancies meeting certain inclusion criteria, while malformations, preterm delivery, SGA, and NICU hospital admissions will be evaluated among pregnancies linked to at least one live-born infant. For each outcome an etiologically relevant window of exposure is determined. The relevant exposure window for the study of spontaneous abortions and stillbirths is uncertain. It could be the week before onset, the period of placentation, or even pre-LMP. We propose to focus on exposure during the three months before pregnancy loss diagnosis; however, several sensitivity analyses will be conducted to consider exposure during other gestational periods. The main etiologically relevant window for the study of congenital malformations is exposure during the first trimester (the period during which organogenesis occurs). Nevertheless, to explore potential effects of exposures before LMP, we will conduct sensitivity analyses to assess risks in women that discontinued within three months of LMP (to assess potential carry over effects); to explore potential effects on some malformations after the first trimester, we will assess the risk associated with exposure during the second or third trimester; and to explore more specific time windows during organogenesis, we will assess the risk associated with exposure for each month within the first trimester. Finally, sensitivity analyses will define as exposed women that received more than one prescription during the first trimester (to reduce misclassification of timing and ensure adherence and enough dose).

For the outcomes of preterm delivery, SGA and NICU hospital admission, three pregnancy exposure periods are potentially relevant: We propose as the primary definition exposures during the three months before delivery because is the period closest to the event and is not affected by length of pregnancy (i.e. longer opportunity for exposure in term than in preterm births). However, these outcomes have been associated with abnormalities in placental development, as well as maternal and fetal factors that develop in late pregnancy. If the medication confers risk of these outcomes by impairing placentation, then the relevant period for exposure may be during the first 20 weeks of gestation (i.e., LMP to day 140 of pregnancy). If the risk for these outcomes occurs as a consequence of late pregnancy exposure, then a later exposure window is more relevant (day 141 of pregnancy to around day 245—the time point at which the outcomes can begin to occur). Since these definitions are sensitive to utilization patterns during pregnancy, we will describe the diagnosis of constipation and the prescription of specific constipation drugs, including prucalopride, from 3 months before LMP until delivery. The relevant exposure windows for each study outcome are summarized in Table 4.1. The same exposure window is applied to prucalopride and reference treatment exposures.

To establish exposure during the etiologically relevant windows requires information on the dates of LMP and delivery so that gestational age and embryogenesis periods can be determined. Because neither gestational length nor LMP date are available in healthcare utilization data, the LMP will be assigned to specific gestational weeks when specific codes are available or, if no specific codes, to be 245 days before the delivery date for preterm deliveries and 270 days before the delivery date for all other pregnancies. Preterm delivery is defined by the presence of International Classification of Diseases Version 9 (ICD-9) codes. This method was validated by our groups for use in claims data and accurately classifies gestational length within 2 weeks for 99% of term deliveries;[20, 21] it was shown to be better than more complicated definitions that included dates of prenatal screening tests like fetal ultrasounds. We have further improved our algorithm by incorporating more specific gestational age codes for premature infants.[22, 23] This algorithm has been demonstrated to have both a sensitivity and specificity (over >96%) for correctly classifying trimester specific exposure status for chronic medications.[24]

More challenging is the timing of pregnancy for pregnancy losses since the exact date of fetal death is often unknown clinically and gestational dating codes are rarely available in health care databases. We define spontaneous abortions as pregnancy losses occurring before 20 weeks and stillbirths as losses occurring at or after 20 weeks of gestation onward. An algorithm for defining the LMP in association with pregnancies that end in spontaneous abortion or stillbirth has been validated in one health care database[23] and applied to MarketScan.[22] This algorithm assigns the median gestational age observed among all spontaneous abortions (12 weeks) and all stillbirths (38 weeks) with some modifications if there are other gestational age indicators available. The LMP estimated from this assignment will be used to apply eligibility criteria and define exposure and baseline covariates.

The main effect measure will be the relative risk for each of the outcomes of interest associated with prucalopride exposure during the etiologically relevant window compared to the reference group. To avoid misclassification of the reference group with maternal use of prucalopride prescribed prior to pregnancy, women with prucalopride dispensed within the three months before LMP will be excluded from the reference group. We will describe the characteristics of prucalopride users, and of women on the primary and secondary reference groups, within the cohort of women with constipation. Within propensity score (PS) strata, women exposed to prucalopride will be compared with prucalopride-unexposed women exposed to other laxatives (primary reference) or just without prucalopride prescriptions (secondary reference).

Table 4.1 Summary of Study Design Including Insurance Eligibility Requirements for Mothers and Offspring, Prucalopride Exposure Windows, Outcome Assessment Windows, and Covariate Assessment Windows

	Insurance eligibility requirement-mother	Insurance eligibility requirement-offspring	Exposure window	Outcome assessment window	Covariate assessment window
Spontaneous abortion	180 days prior to diagnosis	NA	Dispensed in 90 days before diagnosis	NA	180 days prior to diagnosis
Stillbirths	180 days prior to diagnosis	NA	Dispensed in 90 days before diagnosis	NA	180 days prior to diagnosis
Congenital malformations	90 days prior to the LMP to 30 days after delivery	90 days after delivery (unless died)	Dispensed in 1st trimester` LMP to LMP+90	Delivery to 3 months post delivery	90 days prior to the LMP to the end of the 1st trimester
Preterm, small for gestational age, NICU					
PRIMARY	90 days prior to the LMP to 30 days after delivery	30 days after delivery (unless died)	Dispensed delivery-90 to Delivery	Delivery to 30 days post delivery	90 days prior to the LMP to LMP+140
EARLY EXPOSURE	90 days prior to the LMP to 30 days after delivery	30 days after delivery (unless died)	Dispensed LMP to LMP+140	Delivery to 30 days post delivery	90 days prior to the LMP to LMP+140
LATE EXPOSURE	90 days prior to the LMP to 30 days after delivery	30 days after delivery (unless died)	Dispensed LMP+141 to LMP+244 (or delivery)	Delivery to 30 days post delivery	90 days prior to the LMP to LMP+244

4.2 Data Source

All analyses will be conducted using data from IBM Health MarketScan; a commercial health insurer that provides comprehensive medical coverage for members with active policies located throughout the United States. The MarketScan database is one of the largest claims databases, linking paid claims and encounter data to detailed patient information across sites and types of providers, and over time. It contains individual-level demographic and enrollment information, as well as all physician services and hospitalizations and their accompanying diagnoses and procedures. These will be used to define the relevant exposures, covariates, and outcomes in the current study. Diagnostic coding was performed using the Classification of Diseases, 9th revision, Clinical Modification (ICD-9-CM) until 2015. This coding system utilizes six-character alphanumeric codes to describe diagnoses. Starting October 1, 2015 coding was updated to ICD 10. The Current Procedure Terminology (CPT) -4 codes are used to describe medical procedures and physicians' services. A CPT code is a five-digit numeric code. It also contains claims for all filled outpatient medication prescriptions. The pharmacy file provides a history of drug dispensing, it records claims for each filled prescription and refill including the date of dispensing, the drug dispensed, and the quantity dispensed (i.e., the days the supply of drug is anticipated to last, number of prescriptions).

To ensure complete information, the analytic cohorts will be restricted to subjects with full health care and prescription medications coverage throughout the follow up periods. This database has previously been used successfully for perinatal epidemiologic research by us and other groups.[25-29] For details on the creation of pregnancy cohorts in health care claims data see the manuscript by Palmsten et al.[19]

The MarketScan Research Database is the largest nationwide dataset based on commercial health insurance claims, representing more than 100 payers and 25 million covered lives annually. Its sample size is large enough to allow creation of a nationally representative data sample of US residents with employer-provided health insurance. While highly representative of the US, the patterns of use and risk of adverse pregnancy outcomes will be different from populations in publicly insured women within the US, or in developing countries. However, any potential effect of prucalopride will be most likely transportable to other human populations (e.g., thalidomide was teratogenic worldwide).

4.3 Source Population

The source population will consist of pregnancies identified within MarketScan data. As prucalopride became available in April 2019, we will include data from April 2019 onwards including the most recent available data possible at the time of study completion.

In females age 16-44, we will identify all deliveries, spontaneous abortions and stillbirths using inpatient and outpatient delivery-related diagnostic and procedure codes from healthcare utilization claims. For livebirths, the deterministic linkage algorithm we have developed to accurately link mother-infant data files is based on insurance identifier (ID) shared in families and year of birth. Several steps of data cleaning are implemented to ensure accurate linkage and avoid duplication of pregnancies. Strict eligibility criteria are applied to ensure complete claim information for the mother and infant. The cohort is further restricted to women with prescription

benefits. For liveborn deliveries we require mothers to be eligible from at least 90 days prior to the LMP until 30 days after delivery. Infants are required to meet the same eligibility criteria as their mothers for at least 90 days following birth, unless they die before, in which case a shorter eligibility period is allowed.

Requirement of the maternal enrolment period prior to LMP will allow for identification of medication exposures for which the prescription was filled prior to the LMP but whose supply extended into pregnancy. It will also provide accurate ascertainment of comorbid conditions that pre-date pregnancy. The requirement for continuous enrolment throughout pregnancy will allow for complete follow-up and complete ascertainment of medication exposures and claims. Requiring enrolment of infants for at least 90 days following birth will allow ascertainment of nearly all major congenital birth defects.[30] For spontaneous abortions we require mothers to be eligible from 180 days prior to the first code for abortion to 30 days after this diagnosis and for stillbirths eligibility from 180 days prior to the first code for stillbirth to 30 days after this diagnosis is required.

These requirements are crucial to maximize the validity of the study at the potential cost of selecting a study population different from the source. We will present key clinical and demographic characteristics for pregnant women included and excluded (including those not linked to infants) from the study cohort to assess external validity.

4.4 Study Population

Within the source population we will identify a cohort of pregnancies in women with constipation. The study **cohort will be restricted to women with constipation including those with constipation predominant irritable bowel syndrome.** The presence of clinically diagnosed constipation will be based on ICD-10 codes (K58.1, K59.0x, K56.4x or R15.0x) or dispensing of constipation-specific prescription medications recorded during the ascertainment period. Constipation will be defined as ≥1 inpatient or ≥2 outpatient ICD-10 constipation codes on different dates; or ≥1 outpatient code and ≥1 dispensed prescription; or ≥2 dispensed prescriptions on different dates. Within constipation, the codes might allow more **specific etiologies** (e.g. drug-induced, inflammatory bowel syndrome) and measures of **chronicity** (e.g. treatments or codes in prior months). It is important to note that prucalopride is indicated for **chronic idiopathic constipation (ICD10 K59.04)**. However, we will not require this specific code for the primary analyses because prucalopride may be used clinically for related indications (with other codes) and, chiefly, because the coding for claims may not be that specific (i.e., more general codes for constipation may be used) or complete (i.e., in cases of chronic constipation the code might not be coded in all clinical encounters). Rather, in sensitivity analyses, we will aim to use this specific code to further restrict the cohort to pregnancies with chronic idiopathic constipation or prescriptions specifically indicated for it, like prucalopride.

4.4.1 Inclusion Criteria

- Pregnant women with constipation (as defined above).
- Maternal coverage by health care and prescriptions insurance during eligibility period (see Table 4.1): a) Live births: 90 days prior to the last menstrual period until 30 days post-delivery; b) abortions (spontaneous abortions): from 180 days prior to the first code for

abortion to 30 days after this diagnosis; and c) stillbirths: from 180 days prior to the first code for stillbirth to 30 days after this diagnosis.

- For infant outcomes, the cohort will be restricted to pregnancies with linked offspring.
- For major malformations, only pregnancies with livebirths will be included, since information regarding the pathology results from a pregnancy loss or the indication for termination is rarely recorded. In addition, infants are required to have full insurance coverage from delivery to at least 90 days after the delivery, unless the infant died prior to the end of the 90 days, in which case a shorter eligibility period until death is permitted.
- For the analyses of pregnancy losses, the cohort also includes pregnancies ending in spontaneous abortion, stillbirth and any livebirth (linked or unlinked to a delivery).

4.4.2 Exclusion Criteria

- Termination of pregnancy will be identified but excluded from analyses (since malformations are rarely coded and these pregnancies will not be at risk for other outcomes).
- Pregnancies in which prucalopride is dispensed in the 3 months prior to the LMP but not during the first trimester (to ensure that there is not misclassification of the non-exposed).
- Women with opioids dispensed in the 3 months prior to LMP or during the first trimester (to exclude potential opioid-induced constipation) and women with inflammatory bowel diseases (because they are a contraindication).
- For major malformations, the cohort excludes pregnancies with a chromosomal abnormality based on at least one inpatient or outpatient ICD-9 code for 758.xx,759.81-759.83, or 655.1x within 90 days after delivery in the infant and/or maternal claims.
- For major malformations, pregnancies with outpatient exposure to definite teratogens including warfarin, antineoplastic agents, isotretinoin, misoprostol, and thalidomide from LMP through LMP plus 90 days (i.e., days of exposure overlap with 1st trimester).

4.5 Exposure Definitions

4.5.1 Exposure Ascertainment

Maternal exposure to prucalopride and other drugs will be derived from pharmacy dispensing records, with exposure status on any given day based on the dispensing date and, for some analyses, number of doses/days supply. A woman will be considered “unexposed” to a laxative when there is no evidence of prescription filling claim(s) for that agent in the 90 days prior to the exposure window of interest. The 90 days period was chosen because patients can get a 90-day supply for prescriptions that they fill regularly instead of the usual 30-day supply; and patients may extend the use of prescriptions as needed. Therefore, a 90-day wash out window without dispensing claims makes it less likely that the subject is using the medication. For prucalopride, we would exclude from the reference patients with any prescription during this wash out period.

Automated pharmacy dispensing information is usually seen as the gold standard of drug exposure compared to self-reported information[31] or prescribing records in outpatient medical records.[32] Pharmacists fill prescriptions with little room for interpretation, and are reimbursed by insurers on the basis of detailed, complete, and accurate claims submitted electronically.[33, 34] Patient non-response and recall bias are absent from healthcare utilization databases since all

data recording is independent of a patient's memory or agreement to participate in a research study.[35-37]

While the expectation is that patients will generally take medications that they are dispensed, it is possible that in some instances the patients will discontinue the medication or be non-compliant with the prescription. This potential limitation will be addressed in sensitivity analyses in which exposure to prucalopride will be defined by multiple filled prescriptions during the etiologically relevant window, on the assumption that patients who refill the medication are likely to be taking it regularly.

4.5.2 Exposure and Reference Groups

The cohort of pregnancies with constipation will be categorized based on prescriptions into:

- **Prucalopride**, which is the main exposure of interest in this study
- Other laxatives and not prucalopride, which is the primary comparison group
- Untreated (pharmacologically) constipation. "Untreated" constipation will be defined as women with no recorded prescription dispensed for any laxative. It is important to note that these women might be using OTC drugs or old prescriptions stored in their medicine cabinet. The secondary comparison group will comprise these women combined with the "other laxatives" groups into a larger group of women with constipation and not on prucalopride.

The primary exposure of interest is any use of prucalopride during the gestational window of interest. The etiologically relevant period of exposure will be specified a priori and will vary according to the outcome of interest (e.g., first trimester will be the relevant exposure window for the outcome of congenital malformations, see Table 4.1). Timing of pregnancy exposure will be based on the date of the prescription filling together with the gestational timing.

The appropriate comparator depends on factors including knowledge of a medication's position in the treatment pathway (e.g. first, second, third line treatment). This is difficult as prucalopride is relatively new to the market and patterns may change over time. Based on the assumption that prucalopride will be most likely a second- or third-line treatment for women with chronic constipation we will restrict the reference group to **new laxatives** (lubiprostone, linaclotide and plecanatide) in **secondary analyses** (since numbers are expected to be small). Prescription of other laxatives during baseline periods (covariate ascertainment windows) will be considered to control for constipation severity/chronicity, and concomitant use during same etiologically relevant window will also be considered.

If the sample size allows, also as a **secondary analysis**, prucalopride exposure will be further categorized into **combination** with other laxatives or monotherapy. Similarly, if enough sample size accumulates, exposure to prucalopride will be further stratified by the cumulative **dose**.

4.5.3 Timing of Exposure:

Maternal exposure to drugs is derived from pharmacy dispensing records, with exposure status on any given day based on the dispensing date and number of days supply. Defining as exposed

the window covered by days supply maximizes sensitivity but reduces specificity since women may discontinue medication prior to end of days supply. A more conservative definition requires a dispensed prescription during the relevant window, thus increasing specificity since women are more likely to take the medication immediately after filling a prescription. Therefore, we operationalized the ascertainment of exposure based on dispensing of the medication during the windows of interest. However, the supply may cover 30 to 90 days and effects may continue weeks after discontinuation (i.e., carry over effects). For example, a prescription during the week after LMP may be used throughout the first trimester and, even if discontinued at LMP+30 (when pregnancy may be first suspected), effects mediated through metabolic changes may last. Thus, secondary analyses will consider as exposed the days with supplies overlapping the gestational age considered most sensitive.

- **Analyses of spontaneous abortions and stillbirths:** Exposure will be defined by one or more dispensed prescriptions for prucalopride in the 90 days preceding the first coded outcome diagnosis of pregnancy loss compared to the exposure in the cohort at that time window (risk set). Sensitivity analyses will also consider prescriptions from the LMP to day 90 of pregnancy corresponding to the first trimester of pregnancy and from LMP to day 180 of pregnancy corresponding to the first and second trimester of pregnancy (only for stillbirths). The primary reference group will consist of pregnancies without exposure to prucalopride during the corresponding time windows and with one or more dispensed prescriptions for other laxatives during the corresponding time windows.
- **Analyses of major congenital malformations:** Exposure will be defined by one or more dispensed prescriptions for prucalopride from the LMP to day 90 of pregnancy corresponding to the first trimester of pregnancy. The primary reference group will consist of pregnancies without exposure to prucalopride from 90 days prior to the LMP to day 90 of pregnancy and with one or more dispensed prescriptions for other laxatives from the LMP to day 90 of pregnancy. However, the first weeks (LMP to LMP+14) may not be relevant if effects were transient, the sensitive window may be narrower (e.g. LMP+30 to LMP+60), and some malformations may result from exposures after LMP+90. Thus, in sensitivity analyses, we will consider each trimester and each month within the first trimester as alternative windows of exposure.
- **Analyses of small for gestational age, preterm birth and NICU hospital admission:** Exposure will be defined by one or more dispensed prescriptions for prucalopride in the 90 days preceding the delivery (delivery-90 to delivery-1). The primary reference group will consist of pregnancies without exposure to prucalopride during the corresponding time windows and with one or more dispensed prescriptions for other laxatives during the corresponding time windows. Secondary exposure windows include:
    - Early pregnancy exposure: Exposure will be defined by one or more dispensed prescriptions for prucalopride from the LMP to day 140 of pregnancy corresponding to the first 20 weeks of pregnancy. The primary reference group will consist of pregnancies without exposure to prucalopride from 90 days prior to the LMP to day 140 of pregnancy and with one or more dispensed prescriptions for other laxatives from the LMP to day 140 of pregnancy.
    - Late pregnancy exposure: Exposure will be defined by one or more dispensed prescriptions for prucalopride from day 141 of pregnancy to day 245 of pregnancy (or

delivery if sooner) corresponding to the last 20 weeks of pregnancy. The primary reference group will consist of pregnancies without exposure to prucalopride from 90 days prior to the LMP to day 245 of pregnancy and with one or more dispensed prescriptions for other laxatives from day 141 of pregnancy to day 245 of pregnancy.

4.6 Outcome definitions

Outcomes will be defined using previously validated algorithms based on inpatient and/or outpatient diagnoses and procedure claims. We have already completed a large-scale validation study involving chart review designed to optimize the accuracy of algorithms for identifying obstetric and fetal outcomes through ICD-9 codes in claims data. In these studies, our claims based definitions resulted in high PPVs for the outcomes of interest: cardiac malformations (PPV 77.6%), small for gestational age (PPV 86.8%), and preterm delivery (PPV 74.5 %).[38, 39] In a more recent validation study, the PPV was 92% for the improved algorithm for small for gestational age and 86% for non-cardiac congenital malformation as a group (submitted for publication). We are currently validating pregnancy losses, oral clefts and neural tube defects as part of other projects. With these PPV estimates, we can perform a probabilistic bias assessment to explore the effect of outcome misclassification across a range of potential sensitivities and specificities for the outcomes of interest on the relative risk estimates.

Twins or higher order multiples are handled as one pregnancy outcome. For example, if the pregnancy ends in at least one live born infant, the outcome is considered a live born outcome. If either or both twins have a major birth defect, the outcome is considered one major birth defect outcome. Twins are excluded from secondary analyses of preterm delivery and small for gestational age infants (included in primary since constipation is not expected to affect twinning). However, for the consideration of fetal losses (spontaneous abortions or stillbirths), the death of one twin will be counted as fetal loss.

4.6.1 Spontaneous Abortions and Stillbirths

Pregnancy losses will be identified based on ICD-10 codes and CPT codes. We will first identify elective terminations and ectopic pregnancies, and will exclude these pregnancies from the cohort. The outcome of interests will be spontaneous abortions (defined as spontaneous losses before 20 weeks of gestation) and stillbirths (defined as in utero fetal deaths at or after 20 weeks of gestation). The algorithm to include a subject in the pregnancy cohort based on codes for pregnancy losses will require at least 1 code (separated by no more than 30 days) indicative of pregnancy loss.[22, 23, 40] For stillbirths we will search for at least 1 additional code compatible with delivery.

For women with a code for spontaneous abortions and stillbirths, LMP will be estimated by subtracting from the date of end of pregnancy the mean gestational age of occurrence for each outcome based on the literature. Based on this literature and on prior analyses of the data, we expect a baseline risk of over 15% for spontaneous abortions and around 0.4% for stillbirths.[41-43]

4.6.2 Major Congenital Malformations

A major malformation is defined as a structural abnormality with surgical, medical, or cosmetic importance. We will follow child development from LMP to 90 days after delivery. In sensitivity

analyses we will restrict the cohort to infants with at least one year of follow up, unless they died, and expand the follow up to 365 days in the subgroup with sufficient follow up to assess potential for outcome under-ascertainment. While inclusion of outcomes identified during infancy would result in higher risk estimates of malformations, most of the major malformations are diagnosed within weeks of delivery. In the Center for Disease Control (CDC) Metropolitan Atlanta Congenital Defects Program, the frequency changed from around 2.1% for diagnoses within the first week of life to 2.6% if infants were followed until the first birthday. Following the recommendation by the National Birth Defects Prevention Study (NBDPS), birth defects will be sorted and grouped according to the ICD-10.[44] Chromosomal or Mendelian-inherited anomalies, positional deformations, and complications of prematurity will be excluded under the assumption that the etiologies of their malformations are different from those of the remaining cases. Minor anomalies, birth marks, and subclinical anatomic findings by ultrasound will be identified, if recorded, but will not be analyzed since their diagnosis in routine clinical practice and their coding in claims is expected to be incomplete and prone to misclassification. This definition and classification of major structural defects uses the CDC coding manual.[44]

Major malformations will be identified from ICD-10 codes in the child encounter claims (i.e., hospital discharge for the birth or subsequent infant hospitalization) and the mother's claims for codes indicating a birth defect around the child's date of birth (e.g., in obstetric claims). Claims from both the infant and maternal record will be used as claims pertaining to the care of the infant are sometimes applied to the maternal claims in the first few months of life. We will require two codes to define the presence of malformations to exclude cases in which a single mention may be recorded to justify a diagnostic test to rule out a condition. The ascertainment algorithm includes several steps:

1. We first define the presence of one of 13 organ-specific major malformation classes based on the following criteria:
    a. If there is >1 date with a ICD-10 code indicating a malformation documented in the infant records between delivery and delivery +90 days and/or in the maternal records between delivery and delivery +30 days or,
    b. If there is one date with a code for the malformation (as specified above) and a specific surgery code for the correction of a malformation documented in the infant records between delivery and delivery +90 days and/or in the maternal records between delivery and delivery +30 days, or
    c. If there is one date with a code (as specified above) for the malformation group and infant death in the first 30 days.

2. If there is a code for this malformation group in the maternal records between 90 days prior to the LMP and LMP+105 days corresponding to routine screening dates and there are no codes in the infant record between delivery and delivery +90 days (i.e., only maternal codes between delivery and delivery +30 days), then the assumption will be made that the malformation is maternal and we will consider the infant as being unaffected. LMP+105 days is selected because informative (for structural malformations) test results for the fetus could only be available after gestational week 15 during the study period. Codes for a malformation before that time in maternal records more likely belong to maternal diagnoses.

3. If an infant has one or more of the 13 organ specific malformation classes, then we will consider them as having a major malformation overall.

Major malformations are classified into 13 organ specific classes: Central Nervous System, Eye Anomalies, Ear Anomalies, Cardiovascular Anomalies, Other vascular (non-cardiac), Respiratory malformations, Oral clefts, Gastrointestinal, Genital (male and female), Urinary, Musculoskeletal (no limbs, includes omphalocele and gastroschisis), Limb defects, and Other. As data accumulate we might be able to assess the distribution of specific malformations (secondary outcomes) and to estimate the corresponding relative risks. However, it should be noted that, with small numbers and rare events (in the order of 1 per 1000), attempting to estimate relative risks in the context of multiple comparisons (i.e., 13 malformation classes), would result by design in a very high relative risk for the specific malformation in the occurrence of a first case exposed (with wide confidence intervals (CI)).

In a secondary analysis, we will match prucalopride exposed to the reference group of women in a 1:5 ratio using a nearest neighbor algorithm based on propensity scores. De-identified claims profiles of mothers and infants with the outcome of interest as defined using the algorithms will be generated for all the potential cases in this matched cohort. Expert clinicians will review the profiles (temporally ordered listings of medical encounters with associated diagnosis and procedures code descriptions), blinded to the exposure status, and will "adjudicate" each case as (1) the outcome likely occurred, (2) the outcome probably occurred, or (3) the outcome likely did not occur. Since we strive for high specificity of the outcome definition, the effect of excluding (2) and/or (3) on the relative risk estimates will be assessed.

4.6.3 Preterm Delivery, Small for Gestational Age and NICU Admission

Prematurity (<37 weeks of gestation) accounts for approximately 10 percent of all births.[45] Preterm delivery will be defined by the presence of any inpatient or outpatient codes for preterm in the mother or infant record between delivery and delivery +30 days using a validated algorithm.[18, 19, 20, 21, 44, 45, 46] Note that the algorithms were based on ICD-9 codes but we have mapped ICD 10 to ICD-9 codes and expect similar PPVs. We will distinguish between iatrogenic preterm delivery through Cesarean section and vaginal preterm deliveries in secondary analyses.

Low birth weight can be the result of prematurity or of fetal growth retardation or restriction. Infants with fetal growth retardation are born **small for their gestational age** (SGA, birth weight <10th percentile).[45, 47] It is therefore expected to affect up to 10% of newborns. Small for gestational age will be defined by the presence of ≥1 ICD-10 diagnostic codes in maternal or infant claims from delivery to delivery +30.

NICU admission is defined as admission to the Neonatal Intensive Care Unit. We will use CPT codes in maternal and infant claims to identify NICU admission within 30 days of delivery. In a recent study in MarketScan, the frequency of NICU was also approximately 10%.

4.7 Patient Characteristics

Information on covariates considered for confounding adjustment or stratification are obtained from eligibility files (e.g., maternal age), inpatient and/or outpatient claims for diagnoses and

procedures (e.g., diabetes) and pharmacy dispensing records (e.g., concomitant medications). The presence of transient covariates will be obtained during the hypothesis-specific baseline period. The specific time and duration of the baseline period during which baseline characteristics will be ascertained will depend on the etiologically relevant exposure window (Table 4.2). Covariates will be measured with high sensitivity to optimize confounding control.

In the analyses, we will account for conditions that are expected to confound the association between prucalopride exposure and each of the outcomes of interest. For a condition to be a confounder, it needs to be associated with the exposure (i.e., imbalanced between the exposed and reference groups) and associated with the outcome (i.e., risk factor for the outcome). Potential confounders for the association between prucalopride exposure and the outcomes of interest considered include:

- Medical indications for prucalopride: We will consider proxies for constipation severity (e.g., number of outpatient and inpatient constipation-related diagnoses, overall number of visits to specialist or hospitalizations during the baseline period, number of laxative prescriptions).
- Medical contra-indications for prucalopride: Diarrhea, hyperemesis, dehydration.
- Diseases associated with constipation: Irritable bowel syndrome, hypothyroidism, diabetes, celiac disease, and inflammatory bowel disease.
- Medications that have constipation as a side effect: Opioids (users excluded), diuretics, antidepressants, antihistamines, antispasmodics, anticonvulsants, antiarrythmics, beta-adrenoceptor antagonists, calcium channel blockers, anticholinergics, anti-diarrheals, 5-HT3 receptor antagonists (ondansetron), aluminum antacids, and calcium and iron supplements
- Maternal demographic characteristics: Year of delivery, age, parity, obesity.
- Obstetric characteristics/conditions: Multiple gestation.
- Other comorbid medical conditions that are known or suspected risk factors for the outcomes of interest and proxies for such risk factors: hypertension, polycystic ovaries, depression, epilepsy, infections associated with teratogenic effects (e.g., CMV, varicella, rubella), as well as comorbidity score specific to pregnancy.[48]
- Maternal medications. Suspected teratogenic medications (e.g., specific anticonvulsants), other medication use (antidiabetics, antihypertensive medications), and number of distinct prescription drugs used, excluding laxatives, as a general marker of comorbidity.
- Maternal lifestyle habits: smoking, alcohol and illicit drug use. Of note, these factors are expected to be imperfectly captured in claims data. We will include these under-recorded potential confounders to partially adjust for them and based on the assumption that underreporting will not be differential by exposure group. Residual confounding will be explored in sensitivity analyses.
- Health utilization and prenatal testing (which may be markers of overall maternal health and access to care): number of outpatient and inpatient visits, ultrasound and other prenatal tests.

Table 4.2 Covariates Included in Each of the Analyses and the Associated Covariate Assessment Window

Covariates	Covariate assessment window		
	Congenital malformations & spontaneous abortions	Other outcomes (stillbirths will use early exposure)	
		Early exposure	Late exposure
Maternal demographics characteristics	n/a	n/a	n/a
Indications for Prucalopride (constipation severity) **Chronic comorbid conditions** **Other medication exposures**	90 days pre-LMP to 90 days after LMP	90 days pre-LMP to 140 days after the LMP	90 days pre-LMP to 244 days after the LMP
Markers of healthcare utilization Number of medications, outpatient medical visits, hospital admissions, and emergency department visits	90 days pre-LMP to LMP	90 days pre-LMP to LMP	90 days pre-LMP to 140 days after the LMP
Obstetrical conditions	LMP to delivery	LMP to delivery	LMP to delivery
Potentially teratogenic medication exposures	Days' supply overlapping TI	Not relevant	Not relevant

4.8 Data analysis

We will first characterize the source population and the different subcohorts of interest (i.e., women with constipation on different treatment strategies). We will describe patterns of constipation medications throughout pregnancy to understand utilization. The primary analysis will then estimate the relative risk of pre-specified outcomes for pregnancies exposed to prucalopride during etiologically relevant periods compared to the primary reference group (other laxatives) in women with clinically recorded constipation. Secondary analyses will consider a secondary reference group, variations in the exposure window definition, and alternative outcome definitions; explore monotherapy and cumulative dose effects and test the robustness of the data with multiple sensitivity analyses.

4.8.1 Primary Analysis

The same analytic approach will be followed for each of the pregnancy outcomes, unless otherwise noted and for the primary and secondary analysis reference groups.

We will compare the distributions of socio-demographic, clinical and healthcare utilization characteristics during the relevant baseline period for the prucalopride exposed and reference groups. Balance will be assessed using the standardized mean difference. An absolute standardized difference greater than 0.1 will be considered an indicator for substantial imbalances between the two exposure groups.[49]

Absolute risks for the outcome and unadjusted relative risks with their 95% confidence interval (CI) will be calculated. Although it is unlikely to have many repeated pregnancies in the

relatively low follow up period for this study, we will assess whether use of the robust variance estimator to account for correlations within women with multiple pregnancies appreciably changes the CI. If yes, the robust variance estimator will be used in further analyses. If not, the correlation structures will be omitted from all analyses. Absolute risks, risk differences and relative risks with confidence intervals will be presented graphically when appropriate.

Results will be presented for two levels of adjustment: (i) cohort restricted to women using an active comparator (other laxatives) to control for the potential effect of the underlying illness or factors associated with it, (ii) in addition, using PS stratification and inverse probability (of treatment) weights (IPW) standardization to further control for proxies of severity of the underlying indication and other potential confounders.[50] The goal is to attain balance in important risk factors between the exposed and the reference groups.

The PS will be derived from the predicted probability of treatment estimated in a logistic regression model of exposure, which will contain all covariates without additional variable selection.[50, 51] To reduce residual covariate imbalance we will include interaction terms and nonlinear terms to further improve the covariance balance. Each patient is assigned an estimated probability of exposure ranging from 0 to 1 that reflects the likelihood (rather than the actual assignment) of being prescribed an exposure drug, given all measured characteristics. The PS enables all baseline covariates to be combined into a single score and, in doing so, to preserve the ability to adjust for many potential confounders even when the outcome is rare. Non-overlapping areas of the propensity score distributions for the exposed and reference groups will be trimmed to decrease residual confounding.[52] Propensity score trimming ensures exclusion of patients who will always or never receive therapy because of indications or contraindications, i.e. in whom no treatment effect can be estimated, and focuses the estimation of treatment effects in a population with clinical equipoise. For each analysis, we will report the number of exposed and unexposed women trimmed. Since we a priori apply the appropriate inclusion and exclusion criteria to the study population to identify patients eligible for the treatment (i.e., with the indication and no contraindication), we expect minor trimming. We will create 10 equally sized PS-strata based on the distribution among the prucalopride treated women. If there were insufficient number of exposed to support the specification of 10 strata, we will reduce the number to 5. In the outcome models, the reference observations will be weighted using the distribution of the treated among PS-strata. Adjusted relative risks will be estimated using generalized linear models (SAS PROC GENMOD with a weight statement and log link function and binomial distribution for relative risks), and 95% confidence intervals will be calculated using the normal approximation. Balance of baseline characteristics in this weighted population will be assessed using the absolute standardized difference.[49] For each covariate, we will compute the standardized mean difference within each stratum. If standardized differences greater than 0.1 remain for select characteristics, these covariates will be included directly in the outcome model along with weighting on the PS strata.

The propensity score distributions will be plotted for the prucalopride exposure group as well as the different comparator groups. This will allow an objective assessment of whether one comparator group is more appropriate.

In addition, to estimate the average treatment effect, we will use the distribution of propensity scores in the whole study cohort to calculate Inverse Probability of Treatment Weights (IPTWs). Exposed subjects will be assigned a weight equal to the reciprocal of the propensity score, while reference subjects are assigned a weight equal to the reciprocal of one minus the propensity score. We expect the PS distribution in the cohort to be similar to that in the treated given the application of meaningful inclusion criteria to the study population (like a hypothetical clinical trial would include only patients eligible for the treatment). Thus, results from PS stratification and IPTW standardization are expected to be consistent.

All analyses will be conducted in SAS 9.4.

4.8.2 Secondary Analyses

The pre-specified sensitivity analyses for each outcome are summarized in Table 4.3. Sensitivity analyses will address the following:

- To evaluate the potential misclassification of exposure we will re-define the exposure as the presence of at least two dispensings during the period of interest. We will also conduct sensitivity analyses in which exposure will be defined based on days supply that overlaps with the etiologically relevant time window (to reduce the likelihood of misclassifying as unexposed women who had medication from earlier dispensing available).
- To evaluate the effect of potential outcome misclassification, we will a) restrict the outcomes to inpatient diagnoses only (to increase specificity) and b) consider as a potential case any liveborn with ≥1 malformation code (to increase sensitivity) and infants subsequently excluded from primary analysis (to explicitly identify exclusions). For each secondary classification we will summarize the number of cases by pregnancy exposure and malformation group. In addition, we will assess the impact of excluding outcomes designated as "probably occurred" or "likely did not occur" based on review of claims profiles. Moreover, we will correct primary relative risks for outcome misclassification using sensitivities and specificities consistent with the positive predictive values estimated in the internal validation study.[53, 54]
- To evaluate the potential impact of selection bias due to restriction to live births for the analysis of malformations, we will use the methods proposed by Greenland and Khoury.[55, 56] Briefly, we model a range of non-livebirth frequencies for malformed infants in the unexposed and exposed and then calculate "corrected" relative risk estimates based on these inputs and the adjusted relative risk estimate from the main analysis. As such, we conduct a formal quantification of the potential impact of the selection bias.
- To further reduce confounding, we will expand the baseline period to 6 months before LMP to increase the sensitivity to capture proxies for constipation chronicity. The potential impact of residual confounding by factors not measured in external sources, will be quantified using bias analyses. This can be done by defining the strength (confounder-outcome relative risk) of a hypothetical residual confounder which, if

present, would explain the observed effect across a range of confounder prevalence measures in the exposed and reference groups.

For these pre-specified analyses, no adjustments will be made for multiple comparisons, but all results ("negative" or "non-significant" and "positive" or "significant") will be reported.[57]

Table 4.3 Pre-specified Sensitivity Analyses

Sensitivity Analysis	Rationale
All Outcomes	
• Re-define exposure as having filled ≥2 prescriptions for prucalopride during the etiologically relevant time window	Exposure misclassification
• Redefine exposure as days covered by the prescription that overlap with the aetiologically relevant time window	Exposure misclassification
• Evaluate monthly exposure windows from 90 days before LMP to delivery	Specificity of exposure window
• Evaluate exposure by trimester, including the three months before pregnancy (discontinuers) and the second and third trimesters.	Sensitivity of exposure window
• Evaluate the presence of a dose-response relation by considering doses and number of prescriptions for prucalopride	Assess dose response relationship
• Evaluate prucalopride in different combinations with other laxatives	Assess risks with polytherapy
• Correct relative risks for outcome misclassification using sensitivities and specificities consistent with the positive predictive value estimated in the internal validation study	Outcome misclassification
• Expand baseline period to 180 days before LMP	Confounding by constipation severity
• Quantify sensitivity to unmeasured confounders	Residual confounding
Preterm birth • Analysis will be repeated restricting to singleton pregnancies • Analysis will be repeated within mode of delivery (Cesarean-vaginal)	Specificity of association with spontaneous preterm birth phenotype
SGA • Analysis will be repeated restricting to singleton pregnancies	Specificity of association

4.8.3 Interim Monitoring

The number of prucalopride-exposed pregnancies will be monitored at approximately years 3 and 4 after prucalopride approval by FDA in the US, which corresponds to data available in MarketScan during 2022 and 2023. Monitoring reports will update the predicted study power and will inform the statistical analysis plan. The end of the data collection for secondary data use, defined as the date from which the analytical data set is available for the analysis, is planned for Q2 2024. If, based on projections from 2022-2023 counts, the target study size (>100) is not expected to be achieved by Q2 2025, a decision will be made before end of 2024 to determine whether to terminate the study, extend the study period, or involve additional data sources. If the target study size is achieved, but the number of pregnancies available is not large enough to support all the planned analyses, descriptive or unadjusted analyses will be considered.

4.9 Strengths of the Study Design

Use of the IBM MarketScan Database offers many strengths for pharmacoepidemiologic research, including the very large population-based cohort, reliable assessment of drug exposure, and availability of information on a wide range of potential confounders.

4.9.1 Large, Population-based Cohort

IBM MarketScan is the largest dataset based on commercial health insurance claims in the United States. A major strength of this data source is the ability to track individuals longitudinally while they are employed through the same employer, even if they switch health insurance plans. To ensure comprehensive capture of care, we require that women be eligible for insurance during the baseline, exposure window, and follow up period. We further require that the offspring be eligible for insurance during the relevant follow up period.

4.9.2 Objective Assessment of Drug Exposure

Drug exposure will be defined by filled prescriptions during etiologically relevant periods, and recorded before the outcome was known. This approach is not subject to recall bias.

4.9.3 Detailed Information of Potential Confounders

Claims data contains rich information regarding conditions that can potentially confound the association between drug exposures and pregnancy outcomes (e.g., concomitant medications, health care utilization, comorbidity scores). These can be measured and adjusted for in the analyses in a robust manner. Further, the data allow very careful attention to the issue of confounding by indication.

4.10 Limitations of the Research Methods

The study limitations are those of studies using large healthcare utilization databases, or nationwide registries, and center around the potential for misclassification and selection bias.

4.10.1 Exposure Misclassification

Exposure will be ascertained based on filled prescriptions. Non-compliance with the prescription would result in false positives and reduce the PPV of our exposure variable. Under most

scenarios of non-differential misclassification of exposure, this will bias any potential effect towards the null. To guard against exposure misclassification (i.e., false positives), we will favor specificity over sensitivity in our exposure definition, requiring women to have filled at least one prucalopride prescription during the etiologically relevant window (as opposed to having a medication supply available that overlapped with the exposure window). In sensitivity analyses, we will require women to have filled ≥2 prescriptions (i.e., stricter definition) under the assumption that filling multiple prescriptions increases the likelihood that the medication is being taken as prescribed, and we will explore the associations for women with a medication supply that overlaps with the exposure window (i.e., looser definition). There is no risk of recall bias given the data source used, and no risk of false negatives given that prucalopride is not available over-the-counter.

4.10.2 Outcome Misclassification

Ascertainment of outcomes from coded claims can lead to misclassification. We will use highly specific outcome definitions, since this will result in unbiased estimates of the relative risk as long as the sensitivity is non-differential. We will use validated outcome definitions with high positive predictive value. Moreover, the potential impact of misclassification will be assessed through extensive sensitivity analyses. Regardless of these measures taken, some potential for outcome misclassification remains. If non-differential, it would tend to bias relative risk estimates towards the null.

Regarding pregnancy losses, we will miss spontaneous abortions that occurred before a pregnancy was detected or terminations managed outside the health plan (e.g., by a Planned Parenthood). However, the frequency of spontaneous abortions and stillbirths identified in MarketScan[22] is very similar to distributions reported by other health care systems and National Health Statistics (i.e., livebirths 70-80%; spontaneous abortions 10-15%; stillbirths 0.3-0.6%).[23, 58] Hornbrook et al. conducted a validation study of their algorithm compared with medical records abstraction and demonstrated good agreement on the identification and dating of pregnancy outcomes.[23] Ailes et al applied this algorithm to MarketScan data. We used these algorithms as the basis to develop ours.[22]

Regarding the timing, many early losses do not require the immediate attention of a health care provider, do not require a surgical or medical procedure or hospitalization, or have a slowly evolving clinical course preceding a conclusive diagnosis. Precise information on the date the pregnancy ended and the gestational age at termination is therefore difficult to determine.

4.10.3 Comparator Groups

At present there is uncertainty as to the patterns of prescription constipation medication utilization in pregnancy. It is possible that prucalopride may be more commonly used as a second/third line therapy compared with other laxatives which could result in differences in underlying severity of disease or exposure being more or less likely at certain times in pregnancy. To explore these patterns and to identify the most appropriate comparator, the propensity score distributions of the different exposure groups will be compared. We will also consider specific proxies for constipation severity and chronicity.

4.10.4 Residual Confounding

In a non-randomized setting we will have unmeasured or imperfectly measured confounders, which result in residual confounding. Of most concern is the possibility of confounding by the underlying indication. Restriction to women with clinically recorded constipation, use of an active treatment reference and further PS approaches tend to result in balanced characteristics, including risk factors for adverse pregnancy outcomes. However, there could still be confounding by unmeasured or poorly measured confounders. Information on lifestyle factors contained in administrative data is incomplete (e.g., smoking, obesity, alcohol and drug abuse/dependence) or absent (e.g., BMI), which may confound the observed associations to the extent that these factors are not accounted for through adjustment for factors correlated with them. We will compare the balance for measured characteristics among exposure groups before and after PS stratification. We will also explore our ability to predict treatment and the exchangeability of groups by comparison of the PS distributions of the exposure groups. Noteworthy, there are risk factors not available in claims data (e.g., family history of birth defects) that are unlikely to influence the prescription of prucalopride versus other laxatives; i.e., unlikely to be confounders and unlikely to impact the results whether measured or not.

4.10.5 Selection Bias

Stillbirths and spontaneous losses are typically excluded from pregnancy studies based on electronic health records due to inability to determine the exposure windows accurately. Moreover, their identification does not solve lack of information on fetal anatomy for most pregnancy losses, which will affect the assessment of teratogenicity. Claims databases include birth defects information on pregnancies that result in liveborn infants. Any potential diagnoses from fetal autopsy, when performed, are rarely recorded as claims. This is theoretically problematic for studies of the teratogenic potential of medications as there is no information about stillbirths, spontaneous or therapeutic abortions related to the presence of malformations. If non-livebirth frequencies are the same in both the exposed and reference, then the estimates of relative risk in the analysis based on liveborns would be unbiased under most scenarios. However, if non-livebirth of offspring with malformations occurs with greater frequency among women with exposure compared to the non-exposed (within levels of covariates used for adjustment in the analysis), then the analysis which includes only pregnancies resulting in livebirth would underestimate the relative risk of malformations associated with medication exposure. This would occur if women on prucalopride with a prenatally diagnosed fetal malformation were more or less likely to terminate that pregnancy than women on other laxatives (which seems unlikely). We will perform sensitivity analyses to assess the potential impact of missing non-livebirth on the risk estimate for prucalopride and malformations.

4.10.6 Other Missing Information

Medications or vitamins purchase over-the-counter and illicit drug use are not recorded in the source data. However, prescription vitamin use as well as OTC drugs received with a prescription (e.g., acetaminophen) are recorded, as are codes for drug misuse and abuse. Since prucalopride is only available through prescription and since OTC medications are not expected to be strong confounders, missing information on OTC medications is expected to have very limited impact on the study.

Some covariates of interest including smoking and BMI may be missing. However, it is anticipated that the proportion of missing data will be non-differential across the exposure groups. Given the limited follow up in claims databases, it is not possible to adjust for duration of constipation.

4.10.7 Statistical Power

Despite the large size of the data source, since 1) prucalopride is a newly introduced drug and 2) adverse maternal and congenital malformations (the primary outcome) tend to be rare, we will have limited statistical power to detect small increases in risk.

4.11 Study Size

In feasibility analyses, based on 2011-2015 data, 1.8 million pregnancies were identified. Of these around 5% were electively terminated, 16% were spontaneous abortions and 0.3% ended in a stillbirth. Among completed pregnancies, we anticipate over 200,000 liveborn deliveries per year with women eligible from 90 days prior to the LMP to delivery and full insurance coverage for health care and prescriptions. After linkage with neonates in the same family insurance and full benefits, around 125,000 linked liveborn maternal-infant pairs are anticipated to be included in the cohort per year. We expect over 25% of women having constipation during pregnancy. However, the proportion with prescriptions for laxatives during specific periods is expected to be much lower, probably around 5%. If so, the study population would include 6,250 pregnancies per year in the most restrictive cohorts (linked pairs).

Prucalopride was approved in the US in December 2018 and became available in the USA in April 2019. Therefore, we will use all women exposed during the first trimester or late pregnancy as data accumulate from Q2 2019 onwards. Given the 4 to 5 years of data anticipated, we estimate between 50 and 250 exposed pregnancies in the whole study period during specific gestational windows (e.g. first trimester to study malformations).

We estimated the power to detect significant differences (alpha=0.05, 2-sided) at various numbers of exposed women and levels of relative risk for outcomes assuming a prevalence in the unexposed of 10% (e.g., preterm delivery, spontaneous abortions, SGA, NICU)[41, 42] to 3% (e.g., malformations overall). The most common malformations, i.e. cardiac malformations, are approximately 1%. Only under extreme scenarios would we be able to have the statistical power to evaluate them. We assumed a ratio of 5 for the number of unexposed to exposed women.

Table 4.4 Power to Detect Associations Based on the Number Exposed and Relative Risks

	RR				RR				RR			
Exposed	1.5	2	3	5	1.5	2	3	5	1.5	2	3	5
	RISK IN UNEXPOSED: 10%				**RISK IN UNEXPOSED: 3%**				**RISK IN UNEXPOSED: 1%**			
25	0.14	0.32	0.72	0.99	0.08	0.16	0.33	0.66	0.06	0.10	0.18	0.34
50	0.20	0.52	0.93	1.00	0.10	0.23	0.50	0.89	0.07	0.13	0.26	0.52
100	0.33	0.77	1.00	1.00	0.15	0.35	0.74	0.99	0.09	0.18	0.39	0.75
150	0.44	0.90	1.00	1.00	0.18	0.46	0.87	1.00	0.10	0.22	0.50	0.87
300	0.70	0.99	1.00	1.00	0.29	0.70	0.99	1.00	0.14	0.34	0.73	0.99
450	0.85	1.00	1.00	1.00	0.39	0.84	1.00	1.00	0.18	0.45	0.86	1.00
600	0.93	1.00	1.00	1.00	0.48	0.92	1.00	1.00	0.22	0.54	0.93	1.00

Calculations were done using the "Study Size" tool in Episheet (Rothman KJ. Episheet—spreadsheets for the analysis of epidemiologic data. 29 October 2015. Available at: http://www.krothman.org/episheet.xls.)

For the primary outcome, major congenital malformations, the expected prevalence at birth is 3%. If there is an effect, a sample size of 100 exposed would provide power to detect RRs over 3, 150 exposed to detect a RR over 2.6, and 200 exposed to detect a RR over 2.4. Even with small sample sizes, the boundaries of safety would allow the detection of major teratogenicity (e.g., thalidomide) with 25 exposed enrolled, and of moderate teratogenic effects (e.g., valproate) with 50 exposed enrolled. For secondary outcomes such as spontaneous abortions, with a risk over 10%, with 100 exposed women we would be able to detect a 2-fold increased risk with sufficient statistical power. To detect elevations in risks for outcomes with frequencies around 1% or lower (e.g., cardiac malformations, stillbirths).[43] the number of exposed pregnancies needed would be unrealistically high for the near future.

If there are fewer than 50 exposed pregnancies by 2024 in the MarketScan population, neither this data source nor any other study design will be able to find pregnant women exposed in the population. Please note that this is a new drug (i.e., data accumulated before 2019 does not provide exposed) and the drug is less likely to be prescribed to Medicaid enrollees (i.e., the Medicaid claims databases would not add many exposed). From a public health point of view, if there are no pregnant women exposed, there will be less urgency providing data on pregnancy safety. However, if the total number of exposed is under 100 by Q2 2024: 1) If utilization escalates within MarketScan and predictions estimate sufficient numbers with 1 or 2 years of additional data, we would propose to extend the study. 2) If there is evidence of use in the population and adding 2 more years of data were unlikely to provide at least 100 exposed, we would consider adding another database not overlapping with enrollees captured in Marketscan. 3) If there is no uptake of the drug in the specific population of pregnant women, we will discuss with FDA what to do.

5. PROTECTION OF HUMAN SUBJECTS

This study will be submitted to the Institutional Review Board (IRB) of the Harvard T.H. Chan School of Public Health and Brigham & Women's Hospital. Subject consent will not be necessary for this study since the MarketScan (Thomson Reuters) commercial health insurance database is de-identified and HIPPA compliant.

5.1 Ethical Approval and Subject Consent

The proposed research involves the secondary analysis of previously collected data, therefore there is no recruitment and no informed consent procedure. The electronic claims records are anonymous; the identities of patients are not known.

5.2 Subject Confidentiality

This study proposal and the use of all research databases involved will be reviewed by the Harvard T.H. Chan School of Public Health IRB to ensure ethical treatment of human subjects as well as privacy protections (HIPAA). No work will start before IRB approval. We will request waivers of informed consent and of HIPAA authorizations since no more than minimal risk, informed consent is not practicable, and subject rights are not compromised. Research will involve secondary analysis of previously collected data from the IBM MarketScan Database.

6. MANAGEMENT AND REPORTING OF ADVERSE EVENTS

This study will adhere to the International Society for Pharmacoepidemiology (ISPE) good pharmacoepidemiology guidelines (ref). This is a non-interventional study design which is based on retrospective data collection. This study is designed to provide data on risk for adverse birth outcomes in women exposed to prucalopride during pregnancy, based on aggregate analyses. Adverse effects are not being measured directly in this study. Therefore, Takeda/Shire will only report aggregate findings as study reports, not individual spontaneous reports.

7. REFERENCES

1. Bharucha AE, Pemberton JH, Locke GR, 3rd. American Gastroenterological Association technical review on constipation. *Gastroenterology.* 2013;144(1):218-238.
2. Nelson AD, Camilleri M, Chirapongsathorn S, et al. Comparison of efficacy of pharmacological treatments for chronic idiopathic constipation: a systematic review and network meta-analysis. *Gut.* 2017;66(9):1611-1622.
3. Derbyshire E, Davies J, Costarelli V, Dettmar P. Diet, physical inactivity and the prevalence of constipation throughout and after pregnancy. *Matern Child Nutr.* 2006;2(3):127-134.
4. Wald A. Constipation, diarrhea, and symptomatic hemorrhoids during pregnancy. *Gastroenterology clinics of North America.* 2003;32(1):309-322, vii.
5. Wald A, Van Thiel DH, Hoechstetter L, et al. Effect of pregnancy on gastrointestinal transit. *Digestive diseases and sciences.* 1982;27(11):1015-1018.
6. Werler MM, Mitchell AA, Hernandez-Diaz S, Honein MA, Study NBDP. Use of over-the-counter medications during pregnancy. *American journal of obstetrics and gynecology.* 2005;193(3 Pt 1):771-777.
7. Mitchell AA, Gilboa SM, Werler MM, Kelley KE, Louik C, Hernandez-Diaz S. Medication use during pregnancy, with particular focus on prescription drugs: 1976-2008. *American journal of obstetrics and gynecology.* 2011;205(1):51 e51-58.
8. Heinonen OP, Slone D, Shapiro S. *Birth Defects and drugs in pregnancy.* Littleton, Massachusetts: Publishing Sciences Group; 1977.
9. Mahadevan U. Gastrointestinal medications in pregnancy. *Best practice & research Clinical gastroenterology.* 2007;21(5):849-877.
10. Acs N, Banhidy F, Puho EH, Czeizel AE. Senna treatment in pregnant women and congenital abnormalities in their offspring--a population-based case-control study. *Reprod Toxicol.* 2009;28(1):100-104.
11. Mahadevan U, Kane S. American gastroenterological association institute medical position statement on the use of gastrointestinal medications in pregnancy. *Gastroenterology.* 2006;131(1):278-282.
12. Mahadevan U, Kane S. American gastroenterological association institute technical review on the use of gastrointestinal medications in pregnancy. *Gastroenterology.* 2006;131(1):283-311.
13. Suares NC, Ford AC. Prevalence of, and risk factors for, chronic idiopathic constipation in the community: systematic review and meta-analysis. *The American journal of gastroenterology.* 2011;106(9):1582-1591; quiz 1581, 1592.
14. Keating GM. Prucalopride: a review of its use in the management of chronic constipation. *Drugs.* 2013;73(17):1935-1950.
15. Camilleri M, Kerstens R, Rykx A, Vandeplassche L. A placebo-controlled trial of prucalopride for severe chronic constipation. *The New England journal of medicine.* 2008;358(22):2344-2354.
16. Schneeweiss S, Glynn RJ, Tsai EH, Avorn J, Solomon DH. Adjusting for unmeasured confounders in pharmacoepidemiologic claims data using external information: the example of COX2 inhibitors and myocardial infarction. *Epidemiology.* 2005;16(1):17-24.
17. Wyszynski DF, Carman WJ, Cantor AB, et al. Pregnancy and Birth Outcomes among Women with Idiopathic Thrombocytopenic Purpura. *J Pregnancy.* 2016;2016:8297407.

18. Cooper WO, Hernandez-Diaz S, Gideon P, et al. Positive predictive value of computerized records for major congenital malformations. *Pharmacoepidemiology and drug safety.* 2008;17(5):455-460.
19. Palmsten K, Huybrechts KF, Mogun H, et al. Harnessing the Medicaid Analytic eXtract (MAX) to Evaluate Medications in Pregnancy: Design Considerations. *PloS one.* 2013;8(6):e67405.
20. Margulis AV, Setoguchi S, Mittleman MA, Glynn RJ, Dormuth CR, Hernandez-Diaz S. Algorithms to estimate the beginning of pregnancy in administrative databases. *Pharmacoepidemiology and drug safety.* 2012.
21. Margulis AV, Palmsten K, Andrade SE, et al. Beginning and duration of pregnancy in automated health care databases: review of estimation methods and validation results. *Pharmacoepidemiology and drug safety.* 2015;24(4):335-342.
22. Ailes EC, Simeone RM, Dawson AL, Petersen EE, Gilboa SM. Using insurance claims data to identify and estimate critical periods in pregnancy: An application to antidepressants. *Birth defects research Part A, Clinical and molecular teratology.* 2016;106(11):927-934.
23. Hornbrook MC, Whitlock EP, Berg CJ, et al. Development of an algorithm to identify pregnancy episodes in an integrated health care delivery system. *Health services research.* 2007;42(2):908-927.
24. Li Q, Andrade SE, Cooper WO, et al. Validation of an algorithm to estimate gestational age in electronic health plan databases. *Pharmacoepidemiology and drug safety.* 2013;22(5):524-532.
25. Hanley GE, Mintzes B. Patterns of psychotropic medicine use in pregnancy in the United States from 2006 to 2011 among women with private insurance. *BMC pregnancy and childbirth.* 2014;14:242.
26. Ailes EC, Dawson AL, Lind JN, et al. Opioid prescription claims among women of reproductive age--United States, 2008-2012. *MMWR Morbidity and mortality weekly report.* 2015;64(2):37-41.
27. Patorno E, Bateman BT, Huybrechts KF, et al. Pregabalin use early in pregnancy and the risk of major congenital malformations. *Neurology.* 2017;88(21):2020-2025.
28. MacDonald SC, McElrath TF, Hernandez-Diaz S. Pregnancy Outcomes in Women With Multiple Sclerosis. *Am J Epidemiol.* 2019;188(1):57-66.
29. MacDonald SC, McElrath TF, Hernandez-Diaz S. Use and safety of disease-modifying therapy in pregnant women with multiple sclerosis. *Pharmacoepidemiology and drug safety.* 2019;28(4):556-560.
30. Erickson JD. Risk factors for birth defects: Data from the Atlanta birth defects case-control study. *Teratology.* 1991;43:41-51.
31. West S, Savitz D, Koch G, Strom B, Guess H, Hartzema A. Recall accuracy for prescription medications: self-report compared with database information. *American Journal of Epidemiology.* 1995;142(10):1103-1112.
32. West S, Strom B, Freundlich B, Normand E, Koch G, Savitz D. Completeness of prescription recording in outpatient medical records from a health maintenance organization. *Journal of clinical epidemiology.* 1994;47(2):165-167.
33. Stergachis A. Record linkage studies for postmarketing drug surveillance: data quality and validity considerations. *Drug Intell Clin Pharm.* 1988;22(2):157-161.

34. McKenzie D, Semradek J, McFarland B, Mullooly J, McCamant L. The validity of medicaid pharmacy claims for estimating drug use among elderly nursing home residents: The Oregon experience. *Journal of clinical epidemiology.* 2000;53(12):1248-1257.
35. Fowles JB, Lawthers AG, Weiner JP, Garnick DW, Petrie DS, Palmer RH. Agreement between physicians' office records and Medicare Part B claims data. *Health care financing review.* 1995;16(4):189-199.
36. Glynn RJ, Monane M, Gurwitz JH, Choodnovskiy I, Avorn J. Agreement between drug treatment data and a discharge diagnosis of diabetes mellitus in the elderly. *Am J Epidemiol.* 1999;149(6):541-549.
37. Fisher ES, Whaley FS, Krushat WM, et al. The accuracy of Medicare's hospital claims data: progress has been made, but problems remain. *American journal of public health.* 1992;82(2):243-248.
38. Palmsten K, Huybrechts KF, Kowal MK, Mogun H, Hernandez-Diaz S. Validity of maternal and infant outcomes within nationwide Medicaid data. *Pharmacoepidemiology and drug safety.* 2014.
39. Phiri K, Hernandez-Diaz S, Tsen LC, Puopolo KM, Seeger JD, Bateman BT. Accuracy of ICD-9-CM coding to identify small for gestational age newborns. *Pharmacoepidemiology and drug safety.* 2015;24(4):381-388.
40. Devine S, West S, Andrews E, et al. The identification of pregnancies within the general practice research database. *Pharmacoepidemiology and drug safety.* 2010;19(1):45-50.
41. Mukherjee S, Velez Edwards DR, Baird DD, Savitz DA, Hartmann KE. Risk of miscarriage among black women and white women in a U.S. Prospective Cohort Study. *Am J Epidemiol.* 2013;177(11):1271-1278.
42. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. *The New England journal of medicine.* 1988;319(4):189-194.
43. Reddy UM, Ko CW, Willinger M. Maternal age and the risk of stillbirth throughout pregnancy in the United States. *American journal of obstetrics and gynecology.* 2006;195(3):764-770.
44. Rasmussen SA, Olney RS, Holmes LB, Lin AE, Keppler-Noreuil KM, Moore CA. Guidelines for case classification for the National Birth Defects Prevention Study. *Birth defects research Part A, Clinical and molecular teratology.* 2003;67(3):193-201.
45. Halbreich U. The association between pregnancy processes, preterm delivery, low birth weight, and postpartum depressions--the need for interdisciplinary integration. *American journal of obstetrics and gynecology.* 2005;193(4):1312-1322.
46. Toh S, Mitchell AA, Werler MM, Hernandez-Diaz S. Sensitivity and specificity of computerized algorithms to classify gestational periods in the absence of information on date of conception. *Am J Epidemiol.* 2008;167(6):633-640.
47. McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. *The New England journal of medicine.* 1999;340(16):1234-1238.
48. Bateman BT, Gagne JJ. The Obstetric Comorbidity Index predicts severe maternal morbidity. *BJOG : an international journal of obstetrics and gynaecology.* 2015;122(13):1756.

49. Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. *Statistics in medicine.* 2009;28(25):3083-3107.
50. Braitman L, Rosenbaum P. Rare outcomes, common treatments: analytic strategies using propensity scores. *Annals of internal medicine.* 2002;137(8):693-695.
51. Rosenbaum PRR DB. The central role of the propensity score in observational studies for causal effects. *Biometrika.* 1983;70:41-55.
52. Sturmer T, Rothman KJ, Avorn J, Glynn RJ. Treatment effects in the presence of unmeasured confounding: dealing with observations in the tails of the propensity score distribution--a simulation study. *Am J Epidemiol.* 2010;172(7):843-854.
53. Lash TL, Schmidt M, Jensen AO, Engebjerg MC. Methods to apply probabilistic bias analysis to summary estimates of association. *Pharmacoepidemiology and drug safety.* 2010;19(6):638-644.
54. Fox MP, Lash TL, Greenland S. A method to automate probabilistic sensitivity analyses of misclassified binary variables. *International journal of epidemiology.* 2005;34(6):1370-1376.
55. Greenland S. Basic methods for sensitivity analysis of biases. *International journal of epidemiology.* 1996;25(6):1107-1116.
56. Khoury MJ, Flanders WD, James LM, Erickson JD. Human teratogens, prenatal mortality, and selection bias. *American Journal of Epidemiology.* 1989;130:361-370.
57. Rothman KJ, Greenland S. *Modern Epidemiology.* Second ed. Philadelphia: Lippincott-Raven; 1998.
58. Saraiya M, Green CA, Berg CJ, Hopkins FW, Koonin LM, Atrash HK. Spontaneous abortion-related deaths among women in the United States--1981-1991. *Obstetrics and gynecology.* 1999;94(2):172-176.