A Non-interventional Post-authorization Safety Study (NI-PASS) of Outcomes Associated with the Use of Tacrolimus Around Conception, or During Pregnancy or Lactation Using Data from the Transplant Pregnancy Registry International (TPRI)

Final Study Report

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Study Phase:	4
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Product Reference:	EMEA/H/C/000712 IE/H/0165/001-004 EMEA/H/C/000954
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Sponsor (MAH):	Astellas Pharma Europe B.V. Sylviusweg 62 2333 BE Leiden, The Netherlands
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This study was conducted in accordance with International Council for Harmonisation (ICH) Good Clinical Practice (GCP), including the archiving of essential documents.

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1 ABSTRACT

Title

A Non-interventional Post-authorization Safety Study (NI-PASS) of Outcomes Associated with the Use of Tacrolimus Around Conception, or During Pregnancy or Lactation Using Data from the Transplant Pregnancy Registry International (TPRI).

Rationale and Background

Astellas Pharma Europe B.V. is the marketing authorization holder (MAH) for the medicinal products Advagraf™ and Modigraf™ in the European Union (EU). A different Astellas legal entity in each EU country is the MAH for the medicinal product Prograf™. Hereinafter, in this study report, these products and all generic versions of tacrolimus from other manufacturers are referred to as tacrolimus.

Tacrolimus is a calcineurin-inhibitor immunosuppressant indicated for the prevention of organ rejection in patients receiving allogenic kidney, liver or heart transplant in the EU.

Astellas proposed to update section 4.6 of the tacrolimus summary of product characteristics on pregnancy, recommendations for use of contraception in male and female patients, and breastfeeding, based on publicly accessible data from the TPRI, and sought EMA advice per the Worksharing variation procedure submitted on 04 Dec 2018 (EMEA/H/C/WS1511/G). The EMA raised concerns about using the data from the TPRI for supporting the label update: these data were not stratified according to prospectively- and retrospectively-reported pregnancies, the TPRI included mainly retrospectively-reported pregnancies with potential for recall bias, and pregnancies were not stratified with respect to exposure to MPA, a known teratogen which also increases the risk of spontaneous abortion. Given these limitations in the available data, the EMA requested Astellas to conduct an NI-PASS to assess outcomes associated with the use of tacrolimus around conception, or during pregnancy or lactation using data from this registry.

The objective of this NI-PASS was to evaluate outcomes of pregnancies, and outcomes in children and mothers associated with the use of tacrolimus-containing regimens around conception, or during pregnancy or lactation using data from the TPRI (see below for more details on the TPRI). The NI-PASS protocol (Study F506-PV-0001) and the study design were further discussed with the EMA from 29 Sep 2020 (procedures MEA/H/C/000712/MEA/030 for Advagraf and EMEA/H/C/000954/MEA/022 for Modigraf). The study protocol was approved in Sep 2021.

Research Questions and Objectives

Research Questions

1. What are the outcomes of pregnancies in female transplant recipients using tacrolimus (objectives 1-4)?
2. What are the outcomes in children exposed to tacrolimus via breastfeeding (objective 5)?

3. What are the outcomes of pregnancies fathered by male transplant recipients exposed to tacrolimus (objective 6)?
4. What are the maternal outcomes during pregnancy in female transplant recipients using tacrolimus (objectives 7-8)?

Study Objectives

Primary Objective

1. To estimate prevalence of major malformations (as a combined group) among livebirth children born to female transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy. Similarly, for minor malformations (as a combined group).

Secondary Objectives

2. To describe the distribution of types of malformations within the combined major and minor malformation group among children born to female transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.
3. To estimate prevalence of both spontaneous abortions and stillbirths in female transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.
4. To estimate prevalence of small for gestational age (SGA)/intrauterine growth restriction (IUGR) following exposure to tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.
5. To describe the distribution of adverse events in children who were breastfed while their mothers were using tacrolimus (or alternative immunosuppressants, as a combined group).
6. To estimate prevalence of major malformations among children whose reported biological fathers were transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group). Similarly, for minor malformations (as a combined group).
7. To estimate prevalence of gestational diabetes mellitus (GDM) following exposure to tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.
8. To estimate prevalence of gestational hypertension (GH) and pre-eclampsia following exposure to tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.

Study Design

This was a non-interventional follow-up study analyzing prospectively-reported and retrospectively-reported cases separately. Eligible participants were identified to the TPRI by their healthcare providers or they self-enrolled by contacting the TPRI via the website, by email or by telephone. Data on health and medical history, transplant information, pregnancy,

and postpartum outcomes are collected and recorded in the TPRI database. Prospective cases are recorded in the database when the pregnancy is reported to the registry before the pregnancy outcome is known. Retrospective cases are recorded as those where a pregnancy outcome is already known prior to the cases being reported to the registry. Periodic follow-up of the transplant recipient is conducted via telephone interviews with the recipient and/or transplant centers, and by review of medical records, where available.

Setting

The TPRI is based in the United States, was established in 1991 and has actively collected and analyzed data since then. The TPRI began enrollment from countries outside the US in 2016, and by the end of 2020 approximately 7% of all participants had been enrolled from Argentina, Australia, Canada, Croatia, Czech Republic, Denmark, Finland, France, Greece, Guam, Iceland, Kenya, Kuwait, Mexico, Netherlands, New Zealand, Qatar, Romania, South Africa and Switzerland.

Study Population and Study Size

Study Population

Data from transplant recipients using tacrolimus-containing regimens were extracted, together with data from non-tacrolimus-containing regimens (alternative immunosuppressant group). Date of cut-off for inclusion in the study was 31 Dec 2020. Study protocol was finalized in 2021, with data cleaning, data extraction, data analysis, and report writing in 2022 and 2023.

In total, there were 2905 reported pregnancies, 442 prospectively-reported (15%, 383 tacrolimus, 59 alternative treatments) and 2463 retrospectively-reported (85%, 916 tacrolimus, 1547 alternative treatments). Most of the prospective pregnancies were tacrolimus users (87%, 383), whereas the minority of the retrospective pregnancies were tacrolimus users (37%, 916).

The following exposure cohorts were used for analysis:

- Cohort 1 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or through the end of pregnancy, and where the pregnancy resulted in a livebirth (objectives 1 & 2).
- Cohort 2 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or at any time up to 22 weeks of gestation, and where the pregnancy resulted in any pregnancy outcome (objective 3).

- Cohort 3 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy, and where the pregnancy resulted in a livebirth (objective 4).
- Cohort 4 was defined as breastfed children born to female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during breastfeeding (objective 5).
- Cohort 5 was defined as reported pregnancies associated with biological fathers who were kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the 6-week period prior to conception, without use of MPA during the 90-day period prior to conception, and where the pregnancy resulted in a livebirth (objective 6).
- Cohort 6 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy, and where the pregnancy resulted in any pregnancy outcome (objectives 7 & 8).

Data Sources and Variables

The TPRI is an ongoing research registry focused on assessing the effects of pregnancy on transplant recipients and the effects of immunosuppressant medications on fertility and pregnancy outcomes. The TPRI was established in 1991 and has actively collected data via questionnaires, interviews, and medical records. Participation is voluntary, and it involves providing informed consent and does not involve any travel, testing or treatment. The TPRI registers female transplant recipients who have had post-transplant pregnancies or who are pregnant when they register, and male transplant recipients who have fathered pregnancies following an organ transplantation. All pregnancy outcomes are analyzed, including livebirths, spontaneous abortions (miscarriages), therapeutic abortions (terminations), stillbirths, and ectopic pregnancies. The TPRI also collects follow-up data from parents and children which ultimately may be used to study longer-term effects of post-transplant pregnancy in the transplant recipient and the offspring. Long-term neurodevelopmental outcomes were not available for this study. In addition, the TPRI provides information to transplant recipients contemplating post-transplant parenthood and to the healthcare providers who care for them.

Results

Objective 1. Amongst retrospectively-reported cases, there were 61 major malformations among 1706 livebirths, for a prevalence of 3.6% (4.7% for tacrolimus (95% CI: 3.0-6.9), 3.1% for alternative treatments (95% CI: 2.2-4.2). Amongst prospective cases, numbers were smaller with prevalence of major malformations of 2.0% (7/350; 2.0% for tacrolimus (95% CI: 0.7-4.3), 1.9% for alternative treatments (95% CI: 0.0-10.1). Overall, there were no

marked differences between treatment groups for major malformations. Adjusting for a range of explanatory variables in a logistic regression analysis, for each 1-year increase in maternal age at conception, there was a 6% increased risk of major malformations (odds ratio [OR] 1.06, 95% CI: 1.01-1.11).

Objective 1. There were 12 minor malformations among retrospective cases for a prevalence of 0.7% (12/1706), and also 12 minor malformations among prospective cases for a prevalence of 3.4% (12/350). Among prospective cases, all 12 minor malformations were among tacrolimus users (for a prevalence of 4.0%, 12/297); note the tacrolimus group represented 84.9% of prospective cases (297/350).

Objective 2. The distribution of major and minor malformations was fully described. Tongue tie was predominant among minor malformations.

Objective 3. Among retrospective cases, prevalence of spontaneous abortions was higher among users of tacrolimus (26.2%, 95% CI: 23.0-29.5) than users of alternative treatments (14.4%, 95% CI: 12.7-16.2). The pattern was similar among prospective cases although prevalence tended to be lower and numbers smaller, rendering estimates more imprecise (9.9%, 95% CI: 6.9-13.6, for tacrolimus; 5.4%, 95% CI: 1.1-14.9, for alternative treatments). After adjusting for the range of explanatory variables in the logistic regression analyses, tacrolimus remained associated with spontaneous abortions (OR 1.70, 95% CI: 1.31-2.21). Advanced maternal age (≥ 35 years) was also retained in the final model (OR 1.55, 95% CI: 1.02-2.36).

Objective 3. Among retrospective cases, prevalence of stillbirths was 1.4% for tacrolimus users and 2.2% for users of alternative immunosuppressants with no strong evidence of differences between strata. There was only 1 stillbirth reported among the prospective cases. After adjusting for explanatory variables in the logistic regression, neither treatment nor other explanatory variables were associated with risk of stillbirths.

Objective 4. There was no evidence of differences in mean birthweight between users of tacrolimus and alternative treatments. There was a suggestion of a higher prevalence of prematurity and early pre-term birth among tacrolimus users, and larger proportions of tacrolimus users with low birth weight and very low birth weight. For SGA/IUGR, there was a tendency for a lower prevalence in the tacrolimus group than the alternative treatment group. Logistic regression was only conducted for the main outcome for objective 4, i.e., SGA/IUGR, and a decreased risk was reported among tacrolimus users (OR 0.70, 95% CI: 0.57-0.87).

Objective 5. Prevalence of outcomes in breastfed children was not available from the TPRI.

Objective 6. Among prospective cases, numbers were too small to make any inferences about malformations in the offspring of female partners of male transplant recipients (5 livebirths). Among retrospective cases, the prevalence of major malformations was 6.3% in the tacrolimus group (95% CI: 2.1-14.0) and 2.9% in the alternative treatment group (95% CI:

1.8-4.3). For minor malformations, the prevalence was 1.3% (1 of 80; 95% CI: 0.0-6.8) among tacrolimus users, and 0.3% (2 of 738; 95% CI: 0.0-1.0) among those on alternative treatments.

Objective 7. Prevalence of GDM was around 7%, with no evidence of a systematic difference between groups. In the logistic regression analysis, for body mass index (BMI) prior to pregnancy, each unit increase was associated with 3% increased risk of GDM in the combined kidney and liver group (OR 1.03, 95% CI 1.00-1.06), and a 7% increased risk of GDM among liver recipients only (OR 1.07, 95% CI: 1.01-1.14).

Objective 8. Prevalence of GH tended to be systematically higher among kidney recipients than liver recipients, and higher among prospective cases than retrospective cases.

Objective 8. Prevalence of pre-eclampsia tended to be higher among kidney recipients on tacrolimus (37.2% among prospective cases and 34.5% among retrospective cases) than among other groups (range: 18.9%-25.1%). In the logistic regression analysis, tacrolimus use was associated with an increased risk of pre-eclampsia in the kidney group (OR 1.77, 95% CI: 1.39-2.26), but not in the liver group (OR 0.89, 95% CI: 0.53-1.49).

A number of sensitivity analyses were undertaken. Of particular interest, among retrospectively-reported cases delivered prior to 2010, prevalence of major malformations among tacrolimus users was 6.7% (17/253, 95% CI: 4.0-10.5), and for alternative immunosuppressant users 3.1% (36/1148, 95% CI: 2.2-4.3). Among retrospectively-reported cases delivered since 2010, the prevalence of major malformations for tacrolimus users was 2.7% (7/256, 95% CI: 1.1-5.6), and for alternative immunosuppressant users 2.0% (1/49, 95% CI: 0.1-10.9). Given the above trend since 2010, for proportionately more use of tacrolimus and lower prevalence of major malformations among tacrolimus users, the analysis of the full dataset may not optimally reflect the situation in more recent years. However, restricting analyses to more recent data would reduce precision of the estimates.

Conclusions

In this study of kidney and liver transplant recipients, compared to users of non-tacrolimus-containing regimens, there was no evidence of an association between use of tacrolimus-containing regimens during pregnancy and an increased risk of major or minor malformations. There was however an association between use of tacrolimus-containing regimens during pregnancy and an increased risk of spontaneous abortions. Tacrolimus use during pregnancy tended to be associated with prematurity but not mean birth weight nor small for gestational age/intrauterine growth restriction. TPRI did not collect outcomes systematically from children, and so an assessment of the impact of the use of tacrolimus-containing regimens during breastfeeding could not be undertaken. Similarly, pregnancy outcomes associated with male transplant recipients using tacrolimus-containing regimens could not be evaluated since data were limited. There was no strong evidence that use of tacrolimus-containing regimens during pregnancy was associated with gestational

diabetes mellitus or gestational hypertension. The prevalence of pre-eclampsia was found to be higher in the sub-group of kidney recipients using tacrolimus during pregnancy than in kidney recipients using alternative treatments or in liver recipients, however, the reason for this finding is not clear, and results should be interpreted with caution especially given lack of consistency in findings across kidney and liver recipients and substantial changes in diagnostic criteria for pre-eclampsia over the study period.

Overall, results from this study need to be considered in the context of study limitations and potential biases including those associated with the voluntary nature of the study, principally retrospectively-reported data, differences between the treatment groups which could not be controlled for, the design of the study being broadly descriptive, and other factors.

However, even given these limitations, it should be noted that this study used data from the largest and most complete registry of transplant patients systematically collecting information on pregnancy outcomes. The results in general should be reassuring to those transplant recipients who wish to become parents, and in particular the results for the primary objective which showed no evidence of tacrolimus-containing regimens (excluding MPA) or non-tacrolimus-containing regimens (excluding MPA) being associated with elevated prevalence of major malformations.

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2 LIST OF ABBREVIATIONS AND DEFINITION OF KEY TERMS

List of Abbreviations

Abbreviations	Description of Abbreviations
Alt	alternative immunosuppressants
BMI	body mass index
CHMP	Committee for Medicinal Products for Human Use
CI	confidence interval
EMA	European Medicines Agency
ENCePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
EU	European Union
EU PAS register	The European Union Electronic Register of Post-Authorization Studies
GDM	gestational diabetes mellitus
GH	gestational hypertension
MAH	marketing authorization holder
MPA	mycophenolic acid
N/A	not applicable
NI-PASS	non-interventional post-authorization safety study
OR	odds ratio
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2 (i.e., COVID-19)
SGA/IUGR	small for gestational age/intrauterine growth restriction
SLE	systemic lupus erythematosus
Tac	tacrolimus
TPRI	Transplant Pregnancy Registry International

Definition of Key Terms

Terms	Definition of terms
Adverse event	Any untoward medical occurrence in a subject administered a study drug and which does not necessarily have a causal relationship with this treatment.
Estimated date of conception	In the TPRI, conception date is estimated from the reported delivery date and gestational age using the following calculation: Conception date (ddMMMyyyy) = Delivery date (ddMMMyyyy) - ((gestational age in weeks – 2 weeks) *7) days Gestational age is generally estimated from the first day of the last menstrual period or, when the date of the last period is unknown, on the basis of ultrasound findings.
Gestational diabetes mellitus (GDM)	A glucose intolerance resulting in hyperglycemia of variable severity with onset during pregnancy.
Gestational hypertension (GH)	New onset hypertension that occurred after 20 weeks' gestation with blood pressure normalization within six weeks postpartum. [Note: In this study, the working definition of GH was all cases of new onset medication-treated hypertension during pregnancy.]
Major malformation	A life-threatening structural anomaly or one likely to cause significant impairment of health or functional capacity and which needs medical or surgical treatment. [Note: In this study report, the term "malformations" is used to reflect anomalies, birth defects, congenital malformations and malformations.]
Minor malformation	A relatively frequent structural anomaly not likely to cause any medical or cosmetic problems.
Pre-eclampsia	Hypertension & proteinuria after 20 weeks' gestation.
Pregnancy outcome	The outcome of any pregnancy. This can be one of: livebirth, spontaneous abortion (miscarriage), therapeutic abortion (termination), stillbirth or ectopic pregnancy.
Prospectively-reported (or Prospective) case	Case where a pregnancy is reported to the registry before the pregnancy outcome is known.
Retrospectively-reported (or Retrospective) case	Case where a pregnancy outcome is already known prior to the case being reported to the registry.
Small for gestational age (SGA)/Intrauterine growth restriction (IUGR)	SGA/IUGR fetuses or newborns are those smaller in size than normal for their gestational age, most commonly defined as a weight below the 10th percentile for the gestational age. IUGR refers to a condition in which an unborn baby is smaller than it should be because it is not growing at a normal rate inside the womb.
Spontaneous abortion (miscarriage)	Early fetal death (i.e., before 22 completed weeks of gestation).
Stillbirth	Late fetal death (i.e., after 22 completed weeks of gestation).

3 INVESTIGATORS

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TPRI Contact	PPD Transplant Pregnancy Registry International Gift of Life Institute 401 N. 3rd Street Philadelphia, PA 19123, USA Phone: +1 215-599-2078

5 MILESTONES

Milestone	Planned Date	Actual Date
Registration in the EU PAS register	06-Dec-2021	22-Dec-2021
Start of data extraction	10-Jan-2022	03-Jan-2022
End of data extraction	31-Jun-2022	14-Feb-2023
Final report of study results v1.0	31-Oct-2022	20-Jun-2023
Final report of study results v2.0	N/A	03-Oct-2024

6 RATIONALE AND BACKGROUND

Astellas Pharma Europe B.V. is the marketing authorization holder (MAH) for the medicinal products Advagraf™ and Modigraf™ in the European Union (EU). A different Astellas legal entity in each EU country is the MAH for the medicinal product Prograf™. Hereinafter, in this study report, these products are referred to as tacrolimus.

Tacrolimus is a calcineurin-inhibitor indicated for the prophylaxis of organ rejection in patients receiving allogenic kidney, liver or heart transplants in the EU. (Note that Modigraf™ and Prograf™ but not Advagraf™ are authorized for prevention of rejection of heart transplantation in the EU.) Tacrolimus has been approved in the EU for the treatment of allograft rejection resistant to treatment with other immunosuppressive medicinal products. Tacrolimus is available as immediate release capsules, prolonged release capsules, concentrate for solution for infusion and granules for oral suspension.

Tacrolimus inhibits T-lymphocyte activation. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FK506-binding protein 12 (FKBP-12) [Aghdasi et al, 2001]. A complex of tacrolimus-FKBP-12, calcium, calmodulin and calcineurin is then formed and the phosphatase activity of calcineurin is inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells, a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (i.e., immunosuppression) [Tong & Jiang, 2015].

Astellas proposed to update section 4.6 of the tacrolimus summary of product characteristics on pregnancy, recommendations for use of contraception in male and female patients, and breastfeeding, based on publicly accessible data from the TPRI, and sought EMA advice per the Worksharing variation procedure submitted on 04 Dec 2018 (EMEA/H/C/WS1511/G). The EMA raised concerns about using the data from the TPRI for supporting the label update: these data were not stratified according to prospectively- and retrospectively-reported pregnancies, the TPRI included mainly retrospectively-reported pregnancies with potential for recall bias, and pregnancies were not stratified with respect to exposure to MPA, a known teratogen which also increases the risk of spontaneous abortion. Given these limitations in the available data, the EMA requested Astellas to conduct an NI-PASS to assess outcomes

associated with the use of tacrolimus around conception, or during pregnancy or lactation using data from this registry.

The objective of this NI-PASS was to evaluate outcomes of pregnancies, and outcomes in children and mothers associated with the use of tacrolimus-containing regimens around conception, or during pregnancy or lactation using data from the TPRI (see below for more details on the TPRI). The NI-PASS protocol (Study F506-PV-0001), and the study design were further discussed with the EMA from 29 Sep 2020 (procedures MEA/H/C/000712/MEA/030 for Advagraf and EMEA/H/C/000954/MEA/022 for Modigraf). The study protocol was approved in Jul 2021.

7 RESEARCH QUESTIONS AND OBJECTIVES

7.1 Research Questions

1. What are the outcomes of pregnancies in female transplant recipients using tacrolimus (objectives 1-4)?
2. What are the outcomes in children exposed to tacrolimus via breastfeeding (objective 5)?
3. What are the outcomes of pregnancies fathered by male transplant recipients exposed to tacrolimus (objective 6)?
4. What are the maternal outcomes during pregnancy in female transplant recipients using tacrolimus (objectives 7-8)?

7.2 Objectives

The study objectives have been classified as primary and secondary.

7.2.1 Primary Objective

1. To estimate prevalence of major malformations (as a combined group) among livebirth children born to female transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy. Similarly, for minor malformations (as a combined group).

7.2.2 Secondary Objectives

2. To describe the distribution of types of malformations within the combined major and minor malformation group among livebirth children born to female transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.
3. To estimate prevalence of both spontaneous abortions and stillbirths in female transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.
4. To estimate prevalence of small for gestational age (SGA)/intrauterine growth restriction (IUGR) following exposure to tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.

5. To describe the distribution of adverse events in children who were breastfed while their mothers were using tacrolimus (or alternative immunosuppressants, as a combined group).
6. To estimate prevalence of major malformations among children whose reported biological fathers were transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group). Similarly, for minor malformations (as a combined group).
7. To estimate prevalence of gestational diabetes mellitus (GDM) following exposure to tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.
8. To estimate prevalence of gestational hypertension (GH) and pre-eclampsia following exposure to tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.

8 AMENDMENTS AND UPDATES TO THE PROTOCOL AND FINAL STUDY REPORT

Protocol v3.0 (dated 06 Jul 2021) was the first and only EMA-approved version of the study protocol. The final study report (v1.0) was completed in Jun 2023 and submitted to the EMA in Jun 2023. This revised final study report (v2.0) incorporates corrections to errors identified in the original final study report. These corrections to v2.0 have not changed the discussion or conclusions from the previous (v1.0) report.

9 RESEARCH METHODS

Eligible registry participants were generally identified for the TPRI by their healthcare providers. They could also self-enroll by contacting the TPRI via the website (https://www.transplantpregnancyregistry.org/participation/), by email or by telephone during or after pregnancy. The TPRI requires institutional review board approval for their data collection and analysis of data. Informed consent for participating in the registry was collected from each individual and included an agreement for telephone interviews with the recipients to gather information and to request medical records from parent(s) and child(ren). Data collected include health and medication history, transplant information, pregnancy outcome and postpartum data. Periodic follow-up information for the registry on maternal and child health was sought via telephone interviews with the recipient and transplant centers, and by review of medical records. Transplant recipients and their offspring were followed up to determine any long-term effects of post-transplant pregnancy for the recipient or offspring.

All transplant recipients are offered immunosuppressive therapy irrespective of pregnancy status. To evaluate pregnancy outcomes associated with the use of tacrolimus, prospectively-reported and retrospectively-reported cases were analyzed separately in this study. Prospectively-reported cases are those where a pregnancy is reported to the registry before the pregnancy outcome is known. In contrast, retrospectively-reported cases are those

where a pregnancy outcome is already known prior to the cases being reported to the registry, and thus may reflect a more biased sample. In this report, we refer to prospectively-reported cases as prospective cases, and retrospectively-reported cases as retrospective cases.

The tacrolimus group included all those taking tacrolimus-containing regimens, i.e., those taking tacrolimus, irrespective of the use of other immunosuppressants, including any of the following: azathioprine, cyclosporine, everolimus, mycophenolic acid (MPA), prednisone, and sirolimus. The alternative immunosuppressant group included all other regimens. In this report, we refer to the combined group of users of tacrolimus-containing regimens around conception, or during pregnancy or lactation as the tacrolimus group, and the combined group of users of non-tacrolimus-containing regimens around conception, or during pregnancy or lactation as the alternative immunosuppressant group. Note that MPA was an exclusion criterion for cohorts 1, 2 and 5, for both the tacrolimus and alternative immunosuppressant groups.

9.1 Study Design

This was a non-interventional follow-up study analyzing prospective and retrospective cases separately. Data on health and medical history, pregnancy and transplant information, postpartum and pregnancy outcomes were collected and recorded. Prospective cases were recorded in the database as those where the pregnancy was reported to the registry before the pregnancy outcome was known. Retrospective cases were recorded as those where a pregnancy outcome was already known prior to the cases being reported to the registry. Periodic follow-up of maternal and child health was conducted via telephone interviews with the transplant recipient and transplant centers, and by review of medical records.

9.1.1 Endpoints

The ascertainment of all endpoints for the TPRI was performed through a structured interview between the TPRI and the patient, and pregnancy outcomes were validated wherever possible by the patient's healthcare professional. Types of malformation were routinely collected from transplant recipients and validated by healthcare providers. Chromosomal abnormalities, genetic syndromes and cases of multiple anomalies were identified during delivery, hospitalization or also through long-term follow-up.

In this study, livebirth, spontaneous abortion (or miscarriage), stillbirth, SGA/IUGR, GDM, GH and pre-eclampsia were defined by the diagnosis of the practitioner taking care of the transplant patient and/or self-reports by the patient to the TPRI; similarly for the prescription of treatments. The respective method by which these were defined is not recorded by the TPRI and therefore were not considered in this study. Ectopic pregnancies were not included as an endpoint in this study.

9.1.2 Primary Endpoints

Major and minor malformations were the primary endpoints of this study.

9.1.3 Secondary Endpoints

- Pregnancy outcomes: livebirths, spontaneous abortions (miscarriages), stillbirths
- Newborn outcomes: SGA/IUGR
- Breastfeeding outcomes in newborns: any adverse events following exposure to tacrolimus/alternative immunosuppressants postnatally through breastmilk
- Maternal outcomes: GDM, GH, pre-eclampsia

9.2 Alternative Groups

For each of the study cohorts, an alternative immunosuppressant group was defined from those not included in the tacrolimus group (i.e., non-tacrolimus-containing regimens), including users of one or more of the following: azathioprine, cyclosporine, everolimus, MPA, prednisone, and sirolimus.

9.3 Setting

The majority of the study participants were enrolled from the United States. Other participants were enrolled from Argentina, Australia, Canada, Croatia, Czech Republic, Denmark, Finland, France, Greece, Guam, Iceland, Kenya, Kuwait, Mexico, Netherlands, New Zealand, Qatar, Romania, South Africa and Switzerland.

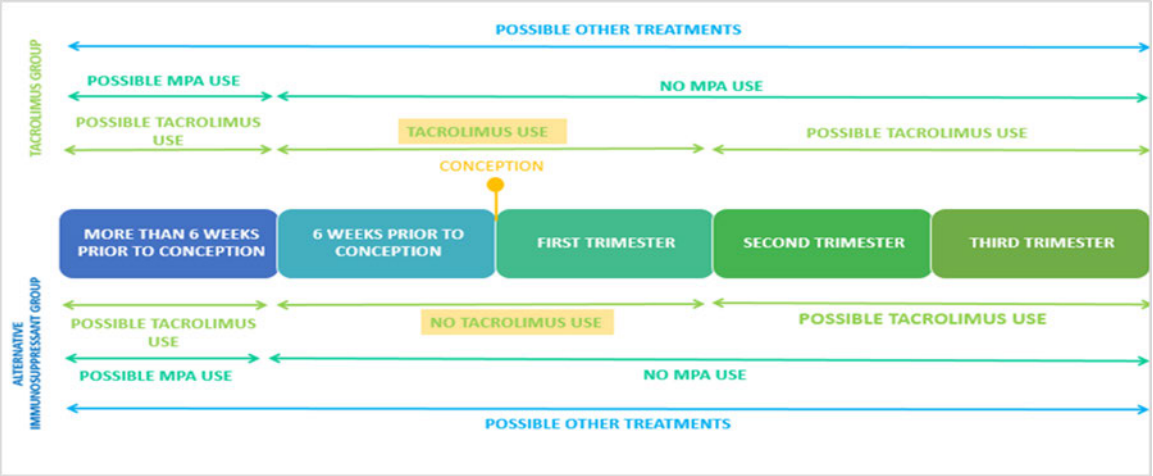
9.4 Patients

For each objective, data from transplant recipients in a defined tacrolimus-exposed group, together with data from a defined alternative immunosuppressant group, were extracted from the TPRI, from initiation of the Registry until 31 Dec 2020. This time period was used for assessment of both exposure and outcomes. The numbers in the Registry reflect number of pregnancies captured by the TPRI. If an individual had multiple pregnancies over several years, each of these pregnancies were included as a separate independent record. The unit of analysis was the overall number of pregnancies, rather than number of individuals.

The different study objectives were addressed using 6 different exposure cohorts, presented in [Figure 1 through Figure 6], respectively:

Cohort 1 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or through the end of pregnancy, and where the pregnancy resulted in a livebirth [Figure 1].

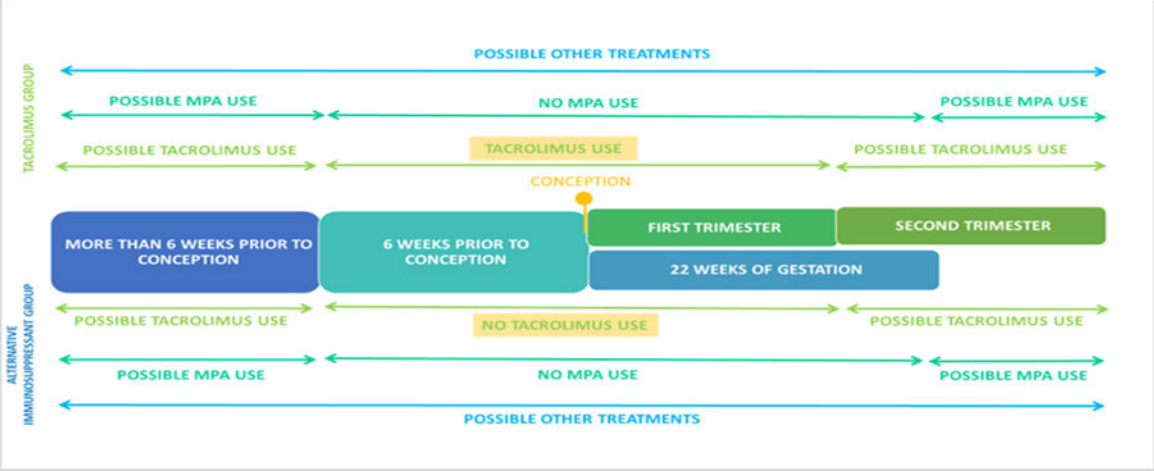
Figure 1 Study Populations (Related to Timing of Use of Immunosuppressants in Female Transplant Recipients) for Objectives 1 & 2 (Cohort 1)



Not to scale; exposure period of interest for tacrolimus and alternative immunosuppressant group highlighted.
MPA: mycophenolic acid

Cohort 2 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or at any time up to 22 weeks of gestation, and where the pregnancy resulted in any pregnancy outcome [Figure 2].

Figure 2 Study Populations (Related to Timing of Use of Immunosuppressants in Female Transplant Recipients) for Objective 3 (Cohort 2)

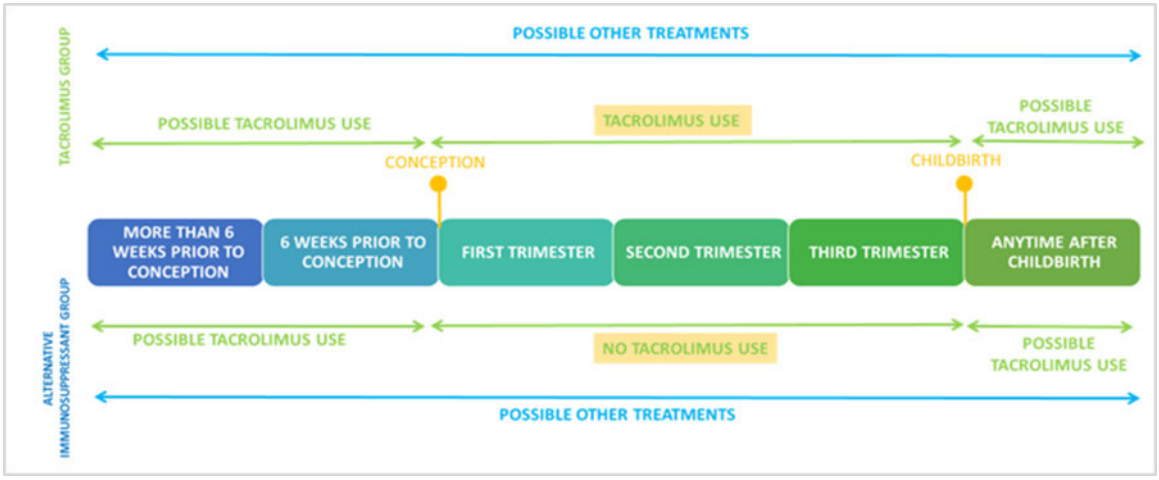


Not to scale; exposure period of interest for tacrolimus and alternative immunosuppressant group highlighted.
MPA: mycophenolic acid

Cohort 3 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as

a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy, and where the pregnancy resulted in a livebirth [Figure 3].

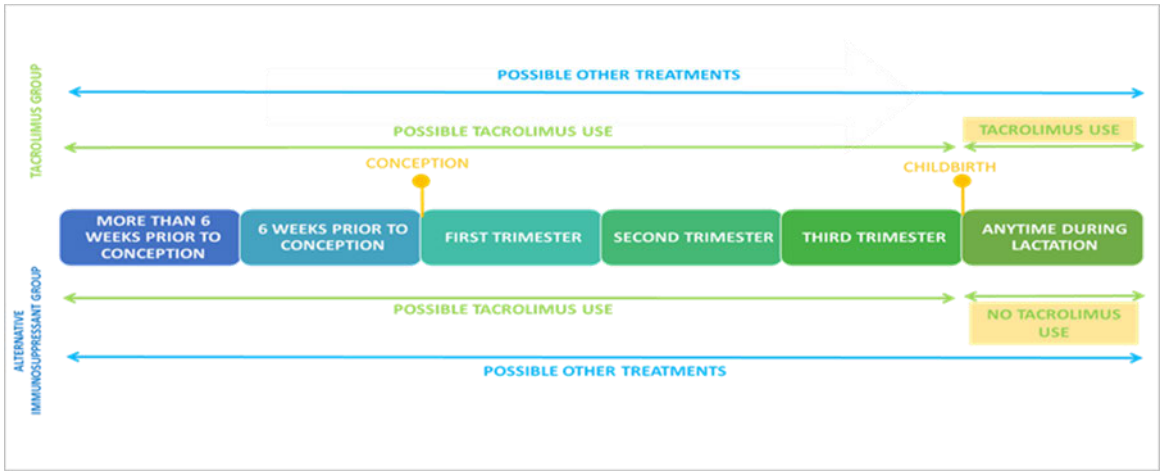
Figure 3 Study Populations (Related to Timing of Use of Immunosuppressants in Female Transplant Recipients) for Objective 4 (Cohort 3)



Not to scale; exposure period of interest for tacrolimus and alternative immunosuppressant group highlighted.

Cohort 4 was defined as breastfed children born to female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during breastfeeding [Figure 4].

Figure 4 Study Populations (Related to Timing of Use of Immunosuppressants in Female Transplant Recipients) for Objective 5 (Cohort 4)

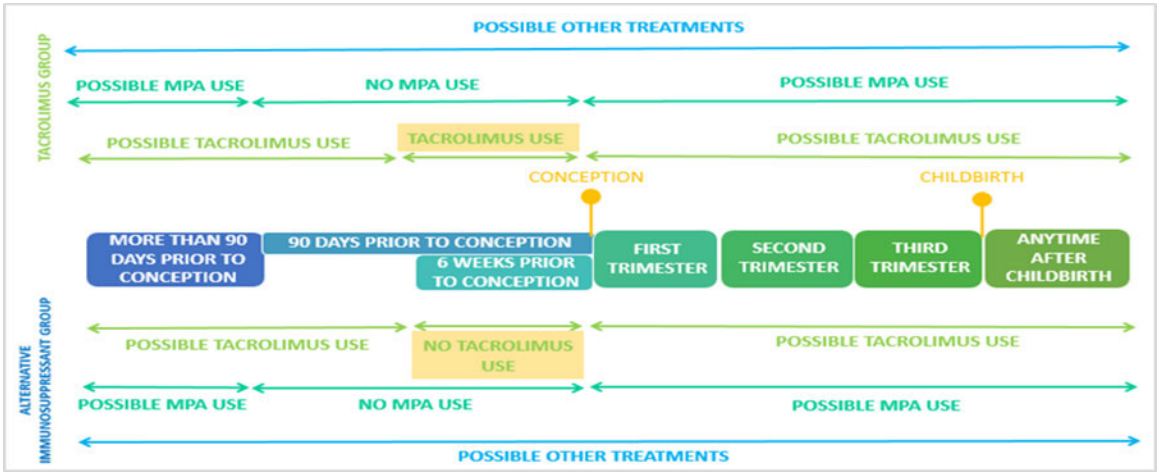


Not to scale; exposure period of interest for tacrolimus and alternative immunosuppressant group highlighted.

Cohort 5 was defined as reported pregnancies associated with biological fathers who were kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined

group, or alternative immunosuppressants, as a combined group) during the 6-week period prior to conception, without use of MPA during the 90-day period prior to conception, and where the pregnancy resulted in a livebirth [Figure 5].

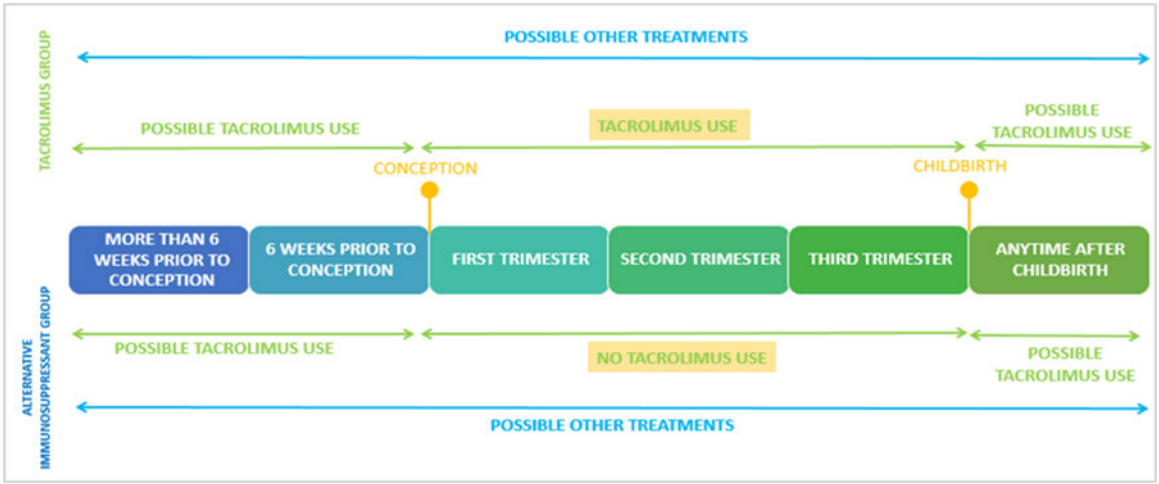
Figure 5 Study Populations (Related to Timing of Use of Immunosuppressants in Male Transplant Recipients) for Objective 6 (Cohort 5)



Not to scale; exposure period of interest for tacrolimus and alternative immunosuppressant group highlighted.
MPA: mycophenolic acid

Cohort 6 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy, and where the pregnancy resulted in any pregnancy outcome [Figure 6].

Figure 6 Study Populations (Related to Timing of Use of Immunosuppressants in Female Transplant Recipients) for Objectives 7 & 8 (Cohort 6)



Not to scale; exposure period of interest for tacrolimus and alternative immunosuppressant group highlighted.

The link between objectives, cohorts and figures are summarized in [Table 1] below.

Table 1 Association of Study Objectives with Cohorts and Figures

Type of objective	Objective No.	Cohort No.	Figure No.
Primary	1	1	1
Secondary	2	1	1
	3	2	2
	4	3	3
	5	4	4
	6	5	5
	7	6	6
	8	6	6

9.4.1 Inclusion Criteria

All transplant recipients exposed to tacrolimus (or alternative immunosuppressants, as a combined group) during the study period and their offspring exposed to tacrolimus (or alternative immunosuppressants, as a combined group) in utero and/or in the postnatal period through breastfeeding were included in the study.

9.4.2 Exclusion Criteria

Exclusion criteria were cohort dependent. In particular, for a number of cohorts (1, 2 and 5), use of MPA was an important exclusion criterion, given its known association with malformations and spontaneous abortions [Khan et al, 2022].

9.4.3 Discontinuation Criteria

Censoring criteria were:

- Death
- Lost to follow-up
- Withdrawal of consent

9.5 Variables

9.5.1 Exposure Definition and Measurement

9.5.1.1 Use of Study Drug and Alternative Immunosuppressants

9.5.1.1.1 Tacrolimus

Tacrolimus use was defined as the transplant recipient taking tacrolimus as described in each objective (see [Figure 1 through Figure 6]). In the TPRI, patients self-report their drug treatment use. For children, exposure to tacrolimus was defined as exposure in utero and/or exposure through breastfeeding. Tacrolimus was usually used continuously by transplant recipients from the time of transplantation through conception and pregnancy.

For three cases, the subjects switched from the alternative treatment group to the tacrolimus group during the specified exposure period, and for one case the subject switched from the tacrolimus group to the alternative treatment group during the specified exposure period. For analyses, all four cases were included in the tacrolimus group.

9.5.1.1.2 Alternative immunosuppressants

Use of alternative immunosuppressants is described in each of the cohort definitions (see [Figure 1 through Figure 6]).

9.5.2 Primary Variables

Dependent Variables

- Major malformation
- Minor malformation
- Spontaneous abortion
- Stillbirth
- SGA/IUGR
- GDM
- GH
- Pre-eclampsia

Explanatory Variables

The following variables were considered for the regression analyses. All variables were recorded at the time of first entry into the registry and reflect a baseline at time of conception, unless otherwise stated. It was assumed that the occurrence of these risk factors was not as a result of exposure to tacrolimus or other immunosuppressants.

- Age of mother at conception (for pregnancies associated with female recipients)
- Age of father at conception (for pregnancies associated with male recipients)
- Pre-transplant pregnancies (for pregnancies associated with female recipients)
- BMI prior to pregnancy
- Systemic lupus erythematosus (SLE)
- Type of conception (natural, assisted)
- Diabetes (at time of entry into the TPRI)
- Hypertension (at time of entry into the TPRI)
- History of pre-eclampsia
- Maternal or paternal history of birth defects
- Known genetic disease
- Advanced maternal age (35 years or older at conception)
- Type of transplant (kidney or liver)
- Date of transplant (to give assessment of time since transplantation)

Limited information was available for the following variables which were not used in the regression analysis:

- Alcohol consumption
- Smoking
- Maternal Infections
- Concurrent bottle (formula) feeding
- Age of mother at conception (for pregnancies associated with male recipients)
- Age of father at conception (for pregnancies associated with female recipients)
- Pre-transplant pregnancies (for pregnancies associated with male recipients)
- Comedications

9.5.3 Secondary Variables

Not applicable

9.5.4 Exploratory Variables

Not applicable

9.6 Data Sources and Measurement

The TPRI is an ongoing research study focused on the effects of pregnancy on transplant recipients and the effects of immunosuppressant medications on fertility and pregnancy outcomes. It was established in 1991 and has actively collected data via questionnaires, interviews and medical records to study the outcomes of pregnancies in transplant recipients. Participation is voluntary, and it involves providing informed consent and does not involve any travel, testing or treatment. The TPRI registers female transplant recipients who have had post-transplant pregnancies or who are pregnant when they register, and male transplant recipients who have fathered pregnancies following an organ transplantation. All pregnancy outcomes are documented, including livebirths, spontaneous abortions (miscarriages), therapeutic abortions (terminations), stillbirths and ectopic pregnancies. The TPRI registry also collects follow-up data from patients and children which is intended to be utilized to study longer-term effects of post-transplant pregnancy in the transplant recipient and the offspring. However, long-term neurodevelopmental outcomes are not available. In addition, The TPRI provides information to transplant recipients contemplating post-transplant parenthood and to the healthcare providers who care for them.

9.7 Bias

This is a non-interventional follow-up study analyzing prospective and retrospective cases from the TPRI separately. Firstly, a potential limitation relates to the identification and classification of endpoint events in the data source. This concerns the validation of the maternal and fetal outcomes which were collected both prospectively and retrospectively in the TPRI's database.

Secondly, this study has a relatively small sample for the analysis of prospective cases and therefore retrospective cases were also analyzed to provide additional context. There are limitations to the analysis of retrospective cases since healthy pregnancy outcomes may be either less or more likely to be reported to the registry depending on the context. In order to account for potential outcome ascertainment bias, the results were stratified per data collection method, i.e., prospective and retrospective.

Thirdly, data collected from interviews are prone to reporting bias due to missing or incomplete data from the participants. For example, medication history for diabetes and hypertension are not systematically captured in the registry and thus history of diabetes and hypertension may not be reliably assessed. Similarly, data on exposure via breastmilk only vs both breastmilk and formula fed are not systematically available.

Fourthly, the voluntary nature of participation in the registry may lead to selection bias.

9.8 Study Size

As of 31 Dec 2020, there were 2905 reported pregnancies, 442 prospective (15%, 383 tacrolimus, 59 alternative treatments) and 2463 retrospective (85%, 916 tacrolimus, 1547 alternative treatments). Most of the prospective pregnancies were tacrolimus users (87%, 383), whereas the minority of the retrospective pregnancies were tacrolimus users (37%, 916). See [Table 6] and [Table 7] for further details.

9.9 Data Management

The TPRI data are stored at the database site in Philadelphia, United States. A TPRI appointed statistician oversaw the data extraction and statistical analysis. Statistical Analysis System (SAS®) Software (Version 9.4) was utilized for access to the raw data and to manage the analytic datasets. The data collected by the TPRI include, but are not limited to, the information recommended in the US FDA's guidance document for establishing pregnancy registries (US Dept. of HHS, FDA, CDER and CBER, Guidance for Industries Establishing Pregnancy Exposure Registries, August 2002). The TPRI is conducted in accordance with ethical principles in the Declaration of Helsinki and aligns with International Conference on Harmonization Good Clinical Practice, Good Epidemiology Practices, and applicable regulatory requirements. This study has followed the relevant chapters of the European Network of Centers for Pharmacoepidemiology and Pharmacovigilance (ENCePP) guidelines for methodological standards and the International Conference on Harmonisation guidelines for data management. Security processes are in place to ensure the integrity of all systems and data. Data were kept secure so that they could not be accessed by anyone other than selected study staff. Data were checked in terms of consistency in flow, range of values, units of measurement, and relevance of clinical information before data analysis.

For this non-interventional study using secondary use of data, reporting of adverse events/reactions to authorities in the form of individual case safety reports was not required according to GVP Module VI.C.1.2.1.

9.10 Statistical Methods

The main focus of the statistical analyses was descriptive, with results generally presented as point estimates and exact Clopper-Pearson 95% confidence intervals (CI). Logistic regression modelling was also undertaken. Note that odds ratios (OR) approximate to relative risks for rare outcomes, and "odds" have been described as "risks" in the results and discussion sections, where appropriate.

9.10.1 Analysis of Primary Objective

The primary objective was descriptive, with prevalence presented as a proportion, together with 95% CI.

9.10.2 Analysis of Secondary Objectives

Secondary objectives were also descriptive, with a point estimate and, where appropriate, confidence interval.

9.10.3 Sample Size Justification

The study included the full available sample from the Registry population, after applying specific inclusion and exclusion criteria for each objective.

9.10.4 Statistical Methods Applied to the Study

Prevalence of an event was defined as outlined in the CHMP Guideline on the Exposure to Medicinal Products during Pregnancy: need for post-authorization data (Guideline number: EMEA/CHMP/313666/2005).

For fetal outcomes

Livebirth prevalence (%):

$$\left(\frac{number\ of\ cases\ among\ live\ born\ infants}{number\ of\ live\ born\ infants}\right) x\ 100\%$$

Birth prevalence (%):

$$\left(\frac{number\ of\ cases\ among\ live\ and\ stillborn\ infants}{number\ of\ live\ and\ stillborn\ infants}\right) x\ 100\%$$

Total prevalence (%):

$$\left(\frac{number\ of\ cases\ among\ live\ births, stillbirths\ and\ terminated\ pregnancies}{number\ of\ live\ births, stillbirths\ and\ terminated\ pregnancies}\right) x\ 100\%$$

For maternal outcomes

Prevalence (%) of GDM:

$$\left(\frac{number\ of\ women\ with\ GDM}{All\ women\ with\ the\ potential\ to\ experience\ GDM}\right)\ x\ 100\%$$

Prevalence (%) of GH or pre-eclampsia:

$$\left(\frac{number\ of\ women\ with\ GH\ or\ pre-eclampsia}{All\ women\ with\ the\ potential\ to\ experience\ GH\ or\ pre-eclampsia}\right)\ x\ 100\%$$

Logistic regression models were used to estimate the association between exposure to tacrolimus-containing regimens and the occurrence of a major or minor malformation (i.e., primary outcome of the study) as well as the occurrence of secondary outcomes. Separate models were run to explore each of the stratification factors (kidney or liver), and immunosuppressant regimen (tacrolimus or alternative immunosuppressant group). The multivariate logistic models explored all available covariates as listed in [Section 9.5.2]. The models were built by progressing from univariate models, using "purposeful selection" and keeping in the final model only the (explanatory) variables statistically significant at the 5% level.

9.10.5 Missing Values

Missing Data in Explanatory Variables

When values for explanatory variables were left blank, it was assumed that the true value was 'no' or '0' and was included in models as such. When data points were missing for continuous variables, analyses were conducted omitting the particular cases. The degree of missing data for each of the explanatory variables has been presented.

9.10.6 Changes to the Planned Analysis

Given the available data, in addition to the main descriptive analyses outlined in the protocol and statistical analysis plan, logistic regression analyses were undertaken for objectives 1, 3, 4, 7 and 8, adjusting for explanatory variables in the assessment of the primary and secondary objectives. All multivariate analyses were undertaken among prospectively- and retrospectively-reported cases combined. Separate analyses were undertaken for kidney recipients only, liver recipients only and kidney and liver recipients combined. The multivariate analyses were undertaken separately for statistical significance at the 5% level. Firstly, univariate analyses were carried out for each of the 3 populations (kidney, liver and kidney and liver combined). Secondly, variables significant in the univariate analyses in any of the 3 populations were included in the final multivariate model selection for all

3 populations. Thirdly, results from the final model were presented for the combined kidney and liver population initially. Fourthly, where there were additional meaningful insights from the kidney or liver populations, these are subsequently presented.

Two additional analyses for major malformations were also conducted, which included (i) stillbirths, and (ii) stillbirths and therapeutic abortions (terminations) with gestational age ≥ 12 weeks, since malformations may be associated with stillbirths and therapeutic abortions, and results for livebirths alone would not capture this. For these analyses, the number of additional malformations were added to the numerators and the number of additional stillbirths and therapeutic abortions were added to the denominators.

In addition to results for hypertension presented for new onset hypertension during pregnancy (i.e., gestational hypertension, GH), an additional analysis was undertaken for any hypertension during pregnancy (HTN), which included both new onset hypertension and chronic pre-pregnancy hypertension. In practice, HTN was recorded when there was evidence of use of any pharmacologic treatment for hypertension.

9.11 Quality Control

The study utilized the existing TPRI database that has been used extensively, mainly for descriptive research purposes. The study was executed in line with applicable regulations and guidelines, including but not limited to the ENCePP Guide on Methodological Standards in Pharmacoepidemiology and the Guidelines for Good Pharmacoepidemiology Practices of the International Society of Pharmacoepidemiology as well as the specific TPRI SOPs. All study programs, log files, and output files were stored on the TPRI secure server.

The TPRI's quality documents were used to guide the conduct of the study. These procedures include internal quality audits, rules for secure and confidential data storage, methods to maintain and archive project documents, quality-control procedures for programming, standards for writing analysis plans, and requirements for senior scientific review. Key programming modules written by a study analyst where specified, and independently reviewed by a different analyst. The programming was conducted by a senior statistician. All key study documents, such as the statistical analysis plan and study reports underwent quality-control review, senior scientific review, and editorial review as per the TPRI SOPs.

Unstructured fields in medical records were reviewed to supplement data from structured data fields. For example, in addressing outcomes associated with breastfeeding, information captured in the unstructured "Child Health" field provided some insights. Analysis data sets and program output were checked for accuracy and integrity according to the TPRI SOPs.

None of the extracted data sets contained data that allow identification of subjects included in the study. Each electronic record was pseudo-anonymized and did not contain any personal identifying data.

10 RESULTS

10.1 Patients

10.1.1 Descriptive Data from the Full TPRI Data Set

[Table 2] reports characteristics of all female kidney and liver recipients reported to the TPRI prior to 31 Dec 2020, including those with MPA exposure. There were 2905 reported pregnancies, 442 prospective (15%, 383 tacrolimus, 59 alternative treatments) and 2463 retrospective (85%, 916 tacrolimus, 1547 alternative treatments). Most of the prospective pregnancies were tacrolimus users (87%, 383), whereas the minority of the retrospective pregnancies were tacrolimus users (37%, 916). There were more kidney than liver transplants among both prospective and retrospective cases. Among retrospective kidney cases, there were proportionately fewer tacrolimus users (31%, 594) than among retrospective liver cases (60%, 322). Descriptive statistics, including mean and standard deviation (SD), are provided for the continuous variable 'age of mother at conception', and prevalence (as a percentage) and number of events are provided for the following dichotomous variables: history of a pre-transplant pregnancy, diagnosis of SLE, conception via natural means, maternal history of a birth defect, maternal history of a genetic disease, and maternal age of ≥ 35 years at conception.

In [Table 2], mean maternal age at conception was consistent across groups, ranging from 28.0 to 33.2 years, slightly lower (1-2 years) in the liver than kidney recipients. The proportion with a history of pre-transplant pregnancies tended to be lower among liver recipients, ranging from 17.6% to 23.3% (with an outlier of 0%), than kidney recipients (24.6%-30.8%). The prevalence of SLE in kidney recipients was similar across groups, ranging from 7.5% to 10.5%, and was 0% in the liver recipients. Natural conception was at least 90% in all groups. Maternal history of a birth defect (i.e., the mother herself had a birth defect) was similar across subgroups, ranging from 11.5% to 20.0% with an outlier of 52.6% (10 of 19 women) among prospective cases of liver recipients using alternative treatments. A maternal history of genetic disease (i.e., the mother herself had a genetic disease) tended to be higher among women with liver transplants (16.7%-31.6%) than those with kidney transplants (6.1%-17.5%). Maternal age ≥ 35 years tended to be more common amongst prospective than retrospective cases respectively (18.4%-31.2% and 8.8%-20.5%) and, for both prospective and retrospective cases, maternal age ≥ 35 tended to be more common amongst kidney than liver recipients.

[Table 3] provides similar information to [Table 2] but for male recipients using immunosuppressants who fathered pregnancies, again not excluding MPA exposures. There were 1117 reported pregnancies, 25 prospective and 1092 retrospective. Among the retrospective cases, most were kidney (87%, 949), and among these most were users of alternative treatments (84%, 800). Descriptive statistics (mean, SD) are provided for the continuous variable 'paternal age at conception', and prevalence (as a percentage) and

number of cases for the following dichotomous variables: SLE as a diagnosis, paternal history of a birth defect, and paternal history of a genetic disease.

In [Table 3], the small number of prospective pregnancies does not allow analysis of trends. In the retrospective pregnancies, mean paternal age at conception was similar across subgroups (32.0-35.8 years), as was history of paternal genetic disease (5.0-9.6%).

Table 2 Characteristics of Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 247	Alt n = 40	Tac n = 136	Alt n = 19	Tac n = 383	Alt n = 59	Tac n = 594	Alt n = 1331	Tac n = 322	Alt n = 216	Tac n = 916	Alt n = 1547
Mean age of mother at conception (years)	32.5	33.2	30.8	29.6	31.9	32.1	31.0	29.3	28.7	28.0	30.2	29.2
Min	14.5	24.7	15.9	20.1	14.5	20.1	18.9	16.4	13.2	16.3	13.2	16.3
Max	47.8	44.3	43.6	40.1	47.8	44.3	47.9	48.2	43.4	43.1	47.9	48.2
Median	32.5	32.8	31.1	29.8	31.9	32.0	30.9	29.6	28.7	28.2	30.2	29.3
SD	4.6	4.5	5.5	5.4	5.0	5.0	4.8	5.4	5.8	5.2	5.3	5.4
Pre-transplant Pregnancies (% [n])	30.8 (76)	27.5 (11)	17.6 (24)	0 (0)	26.1 (100)	18.6 (11)	29.8 (177)	24.6 (328)	23.3 (75)	20.4 (44)	27.5 (252)	24.0 (372)
Systemic Lupus Erythematosus (% [n])	10.5 (26)	7.5 (3)	0 (0)	0 (0)	6.8 (26)	5.1 (3)	8.1 (48)	7.6 (101)	0 (0)	0 (0)	5.2 (48)	6.5 (101)
Natural Conception (% [n])	94.3 (233)	90.0 (36)	94.9 (129)	100.0 (19)	94.5 (362)	93.2 (55)	97.3 (578)	97.7 (1300)	97.8 (315)	98.1 (212)	97.5 (893)	97.7 (1512)
Maternal History of Birth Defects (% [n])	12.6 (31)	20.0 (8)	16.9 (23)	52.6 (10)	14.1 (54)	30.5 (18)	12.6 (75)	15.4 (205)	11.5 (37)	15.7 (34)	12.2 (112)	15.4 (239)
Maternal Genetic Disease (% [n])	9.3 (23)	17.5 (7)	25.0 (34)	31.6 (6)	14.9 (57)	22.0 (13)	8.2 (49)	6.1 (81)	22.4 (72)	16.7 (36)	13.2 (121)	7.6 (117)
Advanced Maternal Age (≥ 35 years at conception) (% [n])	31.2 (77)	30.0 (12)	18.4 (25)	21.1 (4)	26.6 (102)	27.1 (16)	20.5 (122)	15.0 (199)	14.6 (47)	8.8 (19)	18.4 (169)	14.1 (218)

Full female TPRI data set at 31 Dec 2020.

Alt: alternative immunosuppressant group; min: minimum; max: maximum; Tac: tacrolimus group; TPRI: Transplant Pregnancy Registry International.

Table 3 Characteristics of Male Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 13	Alt n = 4	Tac n = 8	Alt n = 0	Tac n = 21	Alt n = 4	Tac n = 149	Alt n = 800	Tac n = 70	Alt n = 73	Tac n = 219	Alt n = 873
Mean age of father at conception (years)	35.7	38.3	32.9	N/A	34.6	38.3	33.9	32.0	35.8	34.3	34.5	32.2
Min	26.8	34.4	27.9	N/A	26.8	34.4	20.1	16.2	24.3	17.8	20.1	16.2
Max	43.3	42.8	38.4	N/A	43.3	42.8	49.3	58.5	61.9	47.3	61.9	58.5
Median	34.6	37.9	32.5	N/A	33.2	37.9	33.5	31.3	34.5	34.4	34.1	31.6
SD	4.9	3.5	3.4	N/A	4.5	3.5	5.8	6.2	7.5	6.4	6.4	6.3
Systemic Lupus Erythematosus (% [n])	0 (0)	0 (0)	0 (0)	N/A	0 (0)	0 (0)	0 (0)	0.4 (3)	0 (0)	0 (0)	0 (0)	0.3 (3)
Paternal history of birth defects (% [n])	15.4 (2)	0 (0)	0 (0)	N/A	9.5 (2)	0 (0)	0 (0)	0.9 (7)	0 (0)	0 (0)	0 (0)	0.8 (7)
Paternal genetic disease (% [n])	15.4 (2)	0 (0)	0 (0)	N/A	9.5 (2)	0 (0)	8.1 (12)	5.0 (40)	7.1 (5)	9.6 (7)	7.8 (17)	5.4 (47)

Full Male TPRI Data Set at 31 Dec 2020.

Alt: alternative immunosuppressant group; min: minimum; max: maximum; N/A: not applicable; Tac: tacrolimus group; TPRI: Transplant Pregnancy Registry International.

10.1.2 Missing Data from Full TPRI Data Set

[Table 4] reports the percentage of missing data for all women. Variables with substantial missing data (i.e., > 10%) included: BMI prior to pregnancy (10.5%-15.0% across the prospective cases, and 18.5%-37.6% across the retrospective cases), type of conception (natural, assisted) (21.1%-43.8%), and history of pre-eclampsia (10.5%-20.6% across prospective cases and 25.9%-37.5% across retrospective cases).

[Table 5] reports percentage of missing data for all men. In particular, there were extensive missing data on fathering pre-transplant pregnancies (54.3%-84.8% across retrospective subgroups).

[Table 6] summarizes number of pregnancies and pregnancy outcomes associated with both female and male transplant recipients across the sub-groups.

[Table 7] summarizes number of pregnancies in each of the cohorts.

The number of patients differs between the results' tables as the different tables report on different cohorts of recipients. For descriptive statistics of continuous variables, the mean, median, minimum, maximum, and standard deviation are generally provided.

In exploring potential tacrolimus pregnancy toxicity, given the known increase in spontaneous abortion (miscarriage) and teratogenicity with first trimester exposure to MPA, cohorts 1, 2 and 5, associated with pregnancy outcomes in objectives 1, 2, 3 and 6, excluded recipients who were taking MPA at defined timepoints relative to conception and pregnancy. Cohort 3, associated with pregnancy outcomes in objective 4, did not exclude recipients taking MPA.

For female transplant recipients, cohorts 1 and 3 were limited to those with livebirths. As per [Table 6], pregnancy outcomes exceed the number of pregnancies due to inclusion of multifetal gestations (twins, triplets and other multiples). Results for birth defects were primarily reported for livebirth pregnancy outcomes.

Results for pre-eclampsia (cohort 6, objective 8) were reported only for pregnancies of at least 20 weeks of gestation.

Table 4 Proportion (%) and Number (n) of Missing Data for Explanatory Variables for Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases

Parameter % (n)	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 247	Alt n = 40	Tac n = 136	Alt n = 19	Tac n = 383	Alt n = 59	Tac n = 594	Alt n = 1331	Tac n = 322	Alt n = 216	Tac n = 916	Alt n = 1547
Missing data for age of mother at conception	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (1)	0 (0)	0 (0)	0 (0)	0.1 (1)	0 (0)
Missing data for pre-transplant pregnancies	0.4 (1)	0 (0)	2.2 (3)	0 (0)	1.0 (4)	0 (0)	0.8 (5)	9.8 (130)	2.8 (9)	3.2 (7)	1.5 (14)	8.9 (137)
Missing data for BMI prior to pregnancy	14.6 (36)	15.0 (6)	14.0 (19)	10.5 (2)	14.4 (55)	13.6 (8)	18.5 (110)	37.6 (500)	23.3 (75)	37.5 (81)	20.2 (185)	37.6 (581)
Missing data for Systemic Lupus Erythematosus	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (1)	0.9 (12)	0 (0)	0 (0)	0.1 (1)	0.8 (12)
Missing data for type of conception (natural, assisted)	35.2 (87)	27.5 (11)	39.0 (53)	21.1 (4)	36.6 (140)	25.4 (15)	36.5 (217)	43.8 (583)	29.8 (96)	28.7 (62)	34.2 (313)	41.7 (645)
Missing data for diabetes during pregnancy	0.4 (1)	5.0 (2)	0.7 (1)	0 (0)	0.5 (2)	3.4 (2)	1.2 (7)	0.6 (8)	1.6 (5)	0 (0)	1.3 (12)	0.5 (8)
Missing data for hypertension during pregnancy	0.4 (1)	0 (0)	0.7 (1)	0 (0)	0.5 (2)	0 (0)	1.7 (10)	4.6 (61)	2.2 (7)	0.5 (1)	1.9 (17)	4.0 (62)
Missing data for history of pre-eclampsia	14.2 (35)	17.5 (7)	20.6 (28)	10.5 (2)	16.4 (63)	15.3 (9)	37.5 (223)	29.5 (393)	37.3 (120)	25.9 (56)	37.4 (343)	29.0 (449)
Missing data for maternal history of birth defects	6.1 (15)	0 (0)	4.4 (6)	5.3 (1)	5.5 (21)	1.7 (1)	2.9 (17)	2.7 (36)	9.0 (29)	19.4 (42)	5.0 (46)	5.0 (78)
Missing data for known genetic disease	6.1 (15)	0 (0)	4.4 (6)	5.3 (1)	5.5 (21)	1.7 (1)	2.9 (17)	2.6 (34)	9.0 (29)	19.4 (42)	5.0 (46)	4.9 (76)

Full female TPRI data set at 31 Dec 2020.

Alt: alternative immunosuppressant group; BMI: body mass index; Tac: tacrolimus group; TPRI: Transplant Pregnancy Registry International.

Table 5 Proportion (%) and Number (n) of Missing Data for Explanatory Variables for Male Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases

Parameter % (n)	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 13	Alt n = 4	Tac n = 8	Alt n = 0	Tac n = 21	Alt n = 4	Tac n = 149	Alt n = 800	Tac n = 70	Alt n = 73	Tac n = 219	Alt n = 873
Missing data for age of father at conception	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (1)	0 (0)	0 (0)	0 (0)	0.1 (1)
Missing data for fathered pre-transplant pregnancies	53.8 (7)	25.0 (1)	25.0 (2)	0 (0)	42.9 (9)	25.0 (1)	59.7 (89)	84.8 (678)	54.3 (38)	82.2 (60)	58.0 (127)	84.5 (738)
Missing data for initial disease	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6.0 (9)	54.5 (436)	1.4 (1)	12.3 (9)	4.6 (10)	51.0 (445)
Missing data for paternal history of birth defects	15.4 (2)	0 (0)	0 (0)	0 (0)	9.5 (2)	0 (0)	0 (0)	0.9 (7)	0 (0)	0 (0)	0 (0)	0.8 (7)
Missing data for known genetic disease	15.4 (2)	0 (0)	0 (0)	0 (0)	9.5 (2)	0 (0)	8.1 (12)	5.0 (40)	7.1 (5)	9.6 (7)	7.8 (17)	5.4 (47)

Full male TPRI data set at 31 Dec 2020.

Alt: alternative immunosuppressant group; Tac: tacrolimus group; TPRI: Transplant Pregnancy Registry International.

Table 6 Total Number of Pregnancies and Pregnancy Outcomes for Female and Male Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases

Transplant	Pregnancies in Female Transplant Recipients				Pregnancies Fathered by Male Transplant Recipients			
	Prospective		Retrospective		Prospective		Retrospective	
	Pregnancies	Outcomes†	Pregnancies	Outcomes†	Pregnancies	Outcomes†	Pregnancies	Outcomes†
Kidney								
Tac	247	258	594	609	13	13	149	156
Alt	40	45	1331	1384	4	6	800	815
Liver								
Tac	136	138	322	336	8	8	70	74
Alt	19	19	216	219	0	0	73	76
Kidney + Liver								
Tac	383	396	916	945	21	21	219	230
Alt	59	64	1547	1603	4	6	873	891

Full female and male TPRI data set at 31 Dec 2020.

Alt: alternative immunosuppressant group; Tac: tacrolimus group; TPRI: Transplant Pregnancy Registry International.

† Includes multiple pregnancy outcomes.

Table 7 Total Number of Pregnancies in Each of the Six Cohorts

Cohort	Prospective						Retrospective						Total
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver		
	Tac	Alt	Tac	Alt	Tac	Alt	Tac	Alt	Tac	Alt	Tac	Alt	
1	185	32	104	18	289	50	304	994	191	160	495	1154	1988
2	205	33	120	19	325	52	435	1272	274	212	709	1484	2570
3	224	37	117	18	341	55	386	1017	210	163	596	1180	2172
4	127	23	76	7	203	30	127	100	66	15	193	115	541
5	1	0	4	0	5	0	32	669	41	58	73	727	805
6	247	40	136	19	383	59	594	1331	322	216	916	1547	2905
6 (GDM)	212	37	125	19	337	56	518	1177	273	210	791	1387	2571
6 (GH)	145	23	116	17	261	40	277	700	256	145	533	845	1679
6 (Pre-eclampsia)	226	37	118	18	344	55	397	1050	216	166	613	1216	2228

Alt: alternative immunosuppressant group; GDM: gestational diabetes mellitus; GH: gestational hypertension; Tac: tacrolimus group.

10.2 Objectives 1 & 2

10.2.1 Descriptive Data for Objectives 1 & 2

Objective 1: To estimate prevalence of major malformations (as a combined group) among livebirth children born to female transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy. Similarly, for minor malformations (as a combined group).

Objective 2: To describe the distribution of types of malformations within the combined major and minor malformation group among livebirth children born to female transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.

[Table 8] shows the characteristics of female transplant recipients whose pregnancy resulted in a livebirth and who did not have MPA exposure during the period from 6 weeks prior to conception through childbirth (cohort 1). Compared to [Table 2], this table excludes MPA exposed pregnancies and non-livebirth outcomes (i.e., spontaneous abortions (miscarriages), therapeutic abortions (terminations), stillbirths, and ectopic pregnancies). Characteristics include age at first transplant, age at conception, duration of interval from transplant to conception, and % with pre-transplant pregnancies. In total, there were 339 prospective cases and 1649 retrospective cases.

In [Table 8], mean maternal age in years at transplant tended to be lower among alternative treatments (especially among prospective cases) and tended to be lower among liver (19.6-20.3) than kidney (20.6-24.6) recipients, with an outlier among prospective liver cases using alternative treatments (6.0, n = 18). Similar to [Table 2], mean maternal age at conception ranged from 28.3 to 33.6 years, and tended to be 1 to 2 years lower in the liver than kidney recipients. Mean interval between transplant and conception for retrospective cases ranged between 5.2 and 8.0 years. For prospective cases, the mean interval was more variable (6.6 to 12.3 years, with an outlier of 23.9 years). The proportion of women with a pre-transplant pregnancy ranged from 20.0 to 25.3% among retrospective cases, and varied across prospective cases from 18.3 to 29.7%, with an outlier of 0%.

Table 8 Characteristics of Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 1, Objectives 1 & 2)

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 185	Alt n = 32	Tac n = 104	Alt n = 18	Tac n = 289	Alt n = 50	Tac n = 304	Alt n = 994	Tac n = 191	Alt n = 160	Tac n = 495	Alt n = 1154
Mean (SD) age at first transplant, range (years)	24.6 (6.6) 2.9-40.2	20.6 (6.5) 7.6-34.4	19.6 (11.0) 0.3-40.6	6.0 (7.5) 0.5-27.4	22.8 (8.7) 0.3-40.6	15.3 (9.8) 0.5-34.4	24.6 (6.1) 2.9-44.2	22.9 (6.0) 1.2-42.4	20.2 (8.7) 0.5-42.2	20.3 (7.4) 1.0-37.8	22.9 (7.5) 0.5-44.2	22.6 (6.2) 1.0-42.4
Mean (SD) age at conception, range (years)	32.7 (4.3) 19.2-45.2	33.6 (4.8) 24.7-44.3	31.2 (5.4) 15.9-43.6	29.9 (5.4) 20.1-40.1	32.2 (4.8) 15.9-45.2	32.2 (5.3) 20.1-44.3	31.2 (4.7) 18.9-47.9	29.3 (5.2) 16.4-48.2	28.4 (5.9) 13.2-42.6	28.3 (5.1) 16.3-39.5	30.1 (5.3) 13.2-47.9	29.1 (5.2) 16.3-48.2
Mean (SD) interval from transplant to conception, range (years)	6.6 (4.8) 0.2-22.9	12.3 (7.1) 1.7-27.6	11.7 (8.8) 0.8-35.8	23.9 (4.3) 12.7-30.9	8.4 (7.0) 0.2-35.8	16.5 (8.4) 1.7-30.9	5.2 (3.4) 0.3-18.0	5.5 (4.1) 0.1-22.5	7.9 (6.6) 0.2-27.9	8.0 (6.9) 0.2-30.7	6.3 (5.1) 0.2-27.9	5.8 (4.7) 0.1-30.7
Pre-transplant pregnancies (% [n])	29.7 (55)	21.9 (7)	18.3 (19)	0 (0)	25.6 (74)	14.0 (7)	25.3 (77)	23.7 (236)	25.1 (48)	20.0 (32)	25.3 (125)	23.2 (268)

Cohort 1 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or through the end of pregnancy (see [Figure 1]), and where the pregnancy resulted in a livebirth.

Alt: alternative immunosuppressant group; MPA: mycophenolic acid; Tac: tacrolimus group .

10.2.2 Outcome Data for Objectives 1 & 2

See [Section 10.2.3] Main Results for Objectives 1 & 2.

10.2.3 Main Results for Objectives 1 & 2

10.2.3.1 Objective 1

The prevalence of major and minor malformations among livebirth offspring of female transplant recipients without MPA exposure is shown in [Table 9] (cohort 1). Amongst retrospective cases, there were 61 major malformations among 1706 livebirths (in 1649 women, see [Table 8]), for a prevalence of 3.6% (4.7% for tacrolimus, 3.1% for alternative treatments). Amongst prospective cases, numbers were smaller with prevalence of major malformations of 2.0% (7/350). There were no marked differences between treatment groups. Given the small numbers, prevalence estimates by treatment or transplant type among prospective cases were unreliable. There were 12 minor malformations among retrospective cases for a prevalence of 0.7% (12/1706), and also 12 minor malformations among prospective cases for a prevalence of 3.4% (12/350). Among prospective cases, all 12 minor malformations were among tacrolimus users (4.0%, 12/297), with the tacrolimus group representing 84.9% of prospective cases (297/350) and the alternative immunosuppressant group representing 15.1% (53/350).

Table 9 Prevalence (%) of Major and Minor Malformations Among Livebirth Children Born to Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 1, Objective 1)

Malformation	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 191	Alt n = 35	Tac n = 106	Alt n = 18	Tac n = 297	Alt n = 53	Tac n = 311	Alt n = 1034	Tac n = 198	Alt n = 163	Tac n = 509	Alt n = 1197
% Major (n) 95% CI	1.6 (3) 0.3-4.5	2.9 (1) 0.1-14.9	2.8 (3) 0.6-8.0	0 (0) 0-18.5	2.0 (6) 0.7-4.3	1.9 (1) 0.0-10.1	4.8 (15) 2.7-7.8	3.1 (32) 2.1-4.3	4.6 (9) 2.1-8.5	3.1 (5) 1.0-7.0	4.7 (24) 3.0-6.9	3.1 (37) 2.2-4.2
% Minor (n) 95% CI	3.7 (7) 1.5-7.4	0 (0) 0-10.0	4.7 (5) 1.5-10.7	0 (0) 0-18.5	4.0 (12) 2.1-7.0	0 (0) 0-6.7	1.0 (3) 0.2-2.8	0.7 (7) 0.3-1.4	1.0 (2) 0.1-3.6	0 (0) 0-2.2	1.0 (5) 0.3-2.3	0.6 (7) 0.2-1.2

Cohort 1 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or through the end of pregnancy (see [Figure 1]), and where the pregnancy resulted in a livebirth.

Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); MPA: mycophenolic acid; Tac: tacrolimus group;

10.2.3.2 Objective 2

[Table 10] details the number of each type of malformation (for major and minor malformations combined). Among prospective cases, 10 of 19 were tongue tie malformations. From the 73 malformations among retrospective cases, the most common were: hypospadias (n = 11), duodenal atresia or stenosis (pyloric stenosis) (n = 5), undescended testicle (n = 5), ventricular septal defect (n = 5) and tongue tie (n = 4).

Table 10 Number of Each Type of Malformation (Major and Minor Malformations Combined) Among Livebirth Children Born to Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 1, Objective 2)

All Malformations	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 191	Alt n = 35	Tac n = 106	Alt n = 18	Tac n = 297	Alt n = 53	Tac n = 311	Alt n = 1034	Tac n = 198	Alt n = 163	Tac n = 509	Alt n = 1197
Nervous system												
Neural tube defects	–	–	–	–	–	–	–	–	–	–	–	–
Anencephalus and similar	–	–	–	–	–	–	–	–	–	–	–	–
Encephalocele	–	–	–	–	–	–	–	–	–	–	–	–
Spina bifida	–	–	–	–	–	–	–	–	–	–	–	–
Hydrocephalus	–	–	–	–	–	–	–	–	–	–	–	–
Severe microcephaly	–	–	–	–	–	–	–	–	–	–	–	–
Arhinencephaly/holoprosencephaly	–	–	–	–	–	–	–	–	–	–	–	–
Other-sacral dimple repair	–	–	–	–	–	–	–	1	–	–	–	1
Other-Arnold Chiari malformation	–	–	–	–	–	–	–	1	–	–	–	1
Other-abnormality of corpus callosum	–	–	–	–	–	–	1	–	–	–	1	–
Eye												
Anophthalmos/micropthalmos	–	–	–	–	–	–	–	–	–	–	–	–
Anophthalmos	–	–	–	–	–	–	–	–	–	–	–	–
Congenital cataract	–	–	–	–	–	–	1	–	–	–	1	–
Congenital glaucoma	–	–	–	–	–	–	–	–	–	–	–	–
Other-iris coloboma (b/l)*	1	–	–	–	1	–	–	–	–	–	–	–
Ear, face and neck												
Anotia	–	–	–	–	–	–	–	–	–	–	–	–
Other-deaf	–	–	–	–	–	–	–	–	1	–	1	–
Other - tongue tie*	5	–	5	–	10	–	1	2	1	–	2	2
Other- ear buds*	–	–	–	–	–	–	–	1	–	–	–	1
Congenital heart defects												
Severe CHD	–	–	–	–	–	–	–	–	–	–	–	–
Common arterial truncus	–	–	–	–	–	–	–	–	–	–	–	–
Double outlet right ventricle	–	–	–	–	–	–	–	–	–	–	–	–
Transposition of great vessels	–	–	–	–	–	–	–	1	–	–	–	1
Single ventricle	–	–	–	–	–	–	–	–	–	–	–	–
Ventricular septal defect (VSD)	–	–	–	–	–	–	–	4	–	1	–	5
Atrial septal defect (ASD)	–	–	–	–	–	–	–	–	–	–	–	–
Atrioventricular septal defect (AVSD)	–	–	–	–	–	–	–	–	–	–	–	–

All Malformations	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 191	Alt n = 35	Tac n = 106	Alt n = 18	Tac n = 297	Alt n = 53	Tac n = 311	Alt n = 1034	Tac n = 198	Alt n = 163	Tac n = 509	Alt n = 1197
Tetralogy of Fallot	1	–	–	–	1	–	1	–	–	–	1	–
Tricuspid atresia and stenosis	–	–	–	–	–	–	–	–	–	–	–	–
Ebstein's anomaly	–	–	–	–	–	–	–	–	–	–	–	–
Pulmonary valve stenosis	–	–	–	–	–	–	–	–	–	–	–	–
Pulmonary valve atresia	–	–	–	–	–	–	–	–	–	–	–	–
Aortic valve atresia/stenosis	–	–	–	–	–	–	–	–	–	–	–	–
Mitral valve anomalies	–	–	–	–	–	–	–	–	–	–	–	–
Hypoplastic left heart	–	–	–	–	–	–	–	–	–	1	–	1
Hypoplastic right heart	–	–	–	–	–	–	–	–	–	–	–	–
Coarctation of aorta	–	–	–	–	–	–	–	–	–	–	–	–
Aortic atresia/interrupted aortic arch	–	–	–	–	–	–	–	–	–	–	–	–
Total anomalous pulmonary venous return	–	–	–	–	–	–	–	–	–	–	–	–
PDA as only CHD in term infants (≥ 37 weeks)	–	–	–	–	–	–	–	–	–	–	–	–
PDA (33 weeks) + bicuspid aortic valve w/surgery	–	–	–	–	–	–	–	1	–	–	–	1
Others: Complex malformations-hypoplastic arch, bicuspid aortic valve, coarctation of aorta, VSD	–	–	1	–	1	–	–	–	–	–	–	–
Respiratory												
Choanal atresia	–	–	–	–	–	–	–	–	–	–	–	–
Cystic adenomatous malformation of lung	–	–	–	–	–	–	1	–	–	–	1	–
Oro-facial clefts												
Cleft lip with or without palate	–	1	–	–	–	1	1	–	–	–	1	–
Cleft palate	–	–	1	–	1	–	1	1	–	–	1	1
Digestive system												
Oesophageal atresia with or without tracheo-oesophageal fistula	–	–	–	–	–	–	–	–	–	–	–	–
Duodenal atresia or stenosis (pyloric stenosis)	–	–	–	–	–	–	1	2+	1	1	2	3
Atresia or stenosis of other parts of small intestine	–	–	–	–	–	–	–	–	–	–	–	–
Ano-rectal atresia and stenosis (imperforate anus)	–	–	–	–	–	–	–	1^	1	–	1	1
Hirschsprung's disease	–	–	–	–	–	–	–	1	–	–	–	1
Atresia of bile ducts	–	–	–	–	–	–	–	–	–	–	–	–
Annular pancreas	–	–	–	–	–	–	–	–	–	–	–	–

All Malformations	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 191	Alt n = 35	Tac n = 106	Alt n = 18	Tac n = 297	Alt n = 53	Tac n = 311	Alt n = 1034	Tac n = 198	Alt n = 163	Tac n = 509	Alt n = 1197
Diaphragmatic hernia	–	–	1	–	1	–	–	–	–	–	–	–
Abdominal wall defects												
Gastroschisis	–	–	–	–	–	–	–	–	–	–	–	–
Omphalocele	–	–	–	–	–	–	–	–	–	–	–	–
Urinary												
Bilateral renal agenesis including Potter syndrome	–	–	–	–	–	–	–	–	–	–	–	–
Multicystic renal dysplasia	–	–	–	–	–	–	1	–	–	–	1	–
Congenital hydronephrosis	–	–	–	–	–	–	–	1	–	–	–	1
Bladder exstrophy and/or epispadia	–	–	–	–	–	–	1	–	–	–	1	–
Posterior urethral valve and/or prune belly	–	–	–	–	–	–	–	–	–	–	–	–
Other-hernia	–	–	–	–	–	–	–	–	1	–	1	–
Other-horseshoe kidney	–	–	–	–	–	–	–	–	1	–	1	–
Other-renal/ureteral reflux-required long term abx (> 3 yrs)	–	–	–	–	–	–	–	1	1	–	1	1
Other-ureter problem (no sx)*	–	–	–	–	–	–	–	1	–	–	–	1
Other-UPJ surgery	–	–	–	–	–	–	1	1	–	–	1	1
Other-medullary sponge kidney	–	–	–	–	–	–	–	–	1	–	1	–
Other-unilateral agenesis of kidney	–	–	–	–	–	–	–	2	1	–	1	2
Genital												
Hypospadias	1	–	–	–	1	–	4	5	1	1	5	6
Indeterminate sex	–	–	–	–	–	–	–	–	–	–	–	–
Other-unilateral ovary*	–	–	–	–	–	–	–	1	–	–	–	1
Other-undescended testicle	–	–	–	–	–	–	1	4	–	–	1	4
Other-chordee*	1	–	–	–	1	–	–	–	–	–	–	–
Limb												
Limb reduction defects	–	–	–	–	–	–	–	–	–	–	–	–
Club foot - talipes equinovarus	1	–	–	–	1	–	–	2	–	–	–	2
Hip dislocation and/or dysplasia	–	–	–	–	–	–	–	–	–	–	–	–
Polydactyly	–	–	–	–	–	–	–	1	–	–	–	1
Syndactyly	–	–	–	–	–	–	–	–	–	–	–	–
Other-extra nubs on hands*	–	–	–	–	–	–	1	–	–	–	1	–
Other-absent nail-5th fingernail*	–	–	–	–	–	–	–	1	–	–	–	1
Other-clinodactyly*	–	–	–	–	–	–	–	–	1	–	1	–
Other-extra bone in toes*	–	–	–	–	–	–	1	–	–	–	1	–
Other anomalies/syndromes												
Skeletal dysplasias	–	–	–	–	–	–	–	–	–	–	–	–

All Malformations	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 191	Alt n = 35	Tac n = 106	Alt n = 18	Tac n = 297	Alt n = 53	Tac n = 311	Alt n = 1034	Tac n = 198	Alt n = 163	Tac n = 509	Alt n = 1197
Craniosynostosis	–	–	–	–	–	–	–	1	–	–	–	1
Congenital constriction bands/amniotic band	–	–	–	–	–	–	–	–	–	–	–	–
Situs inversus*	–	–	–	–	–	–	–	–	–	–	–	–
Conjoined twins	–	–	–	–	–	–	–	–	–	–	–	–
Congenital skin disorders*	–	–	–	–	–	–	–	–	–	–	–	–
Vater/Vacterl	–	–	–	–	–	–	–	–	–	–	–	–
Vascular disruption anomalies	–	–	–	–	–	–	–	–	–	–	–	–
Lateral anomalies	–	–	–	–	–	–	–	–	–	–	–	–
Teratogenic syndromes with malformations	–	–	–	–	–	–	–	–	–	–	–	–
Fetal alcohol syndrome	–	–	–	–	–	–	–	–	–	–	–	–
Valproate syndrome	–	–	–	–	–	–	–	–	–	–	–	–
Maternal infections resulting in malformations	–	–	–	–	–	–	–	–	–	–	–	–
Genetic syndromes + microdeletions	–	–	–	–	–	–	–	–	–	–	–	–
Down syndrome + VSD/ASD	–	–	–	–	–	–	–	–	–	1	–	1
Patau syndrome/trisomy 13	–	–	–	–	–	–	–	–	–	–	–	–
Edward syndrome/trisomy 18	–	–	–	–	–	–	–	–	–	–	–	–
Turner syndrome	–	–	–	–	–	–	–	–	–	–	–	–
Klinefelter syndrome	–	–	–	–	–	–	–	–	–	–	–	–
Other-polythelia*	–	–	–	–	–	–	–	1	–	–	–	1
Other-Wiskott Aldrich syndrome	–	–	–	–	–	–	–	1	–	–	–	1

Cohort 1 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or through the end of pregnancy (see [Figure 1]), and where the pregnancy resulted in a livebirth.

abx: antibiotics; Alt: alternative immunosuppressant group; ASD: atrial septal defect; AVSD: atrioventricular septal defect; b/l: bilateral; CHD: congenital heart disease; MPA: mycophenolic acid; PDA: patent ductus arteriosus; sx: symptoms; Tac: tacrolimus group; UPJ: ureteropelvic junction; VSD: ventricular septal defect.

+ 1 individual recorded as duodenal atresia/stenosis also had ureter surgery for hydronephrosis.
^ 1 individual recorded as ano-rectal atresia and stenosis also had clubbed-foot.
* rows with minor malformations.

10.2.4 Additional Results for Objectives 1 & 2

Objective 1

Additional analyses included stillbirths (in addition to livebirths) in the denominator (see [Table 11]). There were only minor differences in the prevalence across subgroups. Further analyses extended this to also include therapeutic abortions (terminations) after ≥ 12 weeks in the denominator (see [Table 12)]. Again there were only minor differences in prevalence across subgroups.

Prevalence of major malformations among livebirths, stillbirths and terminations by maternal age at conception (< 35 or ≥ 35 years) was also explored (see [Table 13]). Numbers were relatively small in the prospective group. There was a suggestion of a trend towards higher prevalence among older mothers in the retrospective group, with prevalence of major malformations in the alternative treatment group for the ≥ 35 year age group of 6.5% (95% CI: 3.3-11.3) and for the < 35 year age group of 3.0% (95% CI: 2.1-4.2).

Adjusting for a range of explanatory variables in the logistic regression analysis (as specified in [Section 9.10.4]), the only variable retained in the model was maternal age at conception where, for each 1-year increase in age at conception, there was a 6% increased risk of major malformations in the combined kidney and liver group (OR 1.06, 95% CI: 1.01-1.11, n = 2056). There was no evidence of a difference between the tacrolimus and alternative immunosuppressant groups (OR 1.10, CI: 0.67-1.81, n = 2056). Consistent with [Table 9], the results of the logistic regression analysis for minor malformations showed a 3.8-fold increased risk in the tacrolimus group, with a relatively wide CI (OR 3.79, 95% CI: 1.56-9.18, n = 2056). No other explanatory variables were retained in the model.

Objective 2

No additional analyses were undertaken.

Table 11 Prevalence (%) of Major and Minor Malformations Among Livebirths and Stillbirths of Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 1 [Extra 1], Objective 1)

Malformation	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 192	Alt n = 35	Tac n = 106	Alt n = 18	Tac n = 298	Alt n = 53	Tac n = 317	Alt n = 1064	Tac n = 202	Alt n = 166	Tac n = 519	Alt n = 1230
% Major (n) 95% CI	1.6 (3) 0.3-4.5	2.9 (1) 0.1-14.9	2.8 (3) 0.6-8.0	0 (0) 0-18.5	2.0 (6) 0.7-4.3	1.9 (1) 0.0-10.1	5.0 (16) 2.9-8.1	3.2 (34) 2.2-4.4	4.5 (9) 2.1-8.3	3.0 (5) 1.0-6.9	4.8 (25) 3.1-7.0	3.2 (39) 2.3-4.3
% Minor (n) 95% CI	3.6 (7) 1.5-7.4	0 (0) 0-10.0	4.7 (5) 1.5-10.7	0 (0) 0-18.5	4.0 (12) 2.1-6.9	0 (0) 0-6.7	0.9 (3) 0.2-2.7	0.7 (7) 0.3-1.4	1.0 (2) 0.1-3.5	0 (0) 0-2.2	1.0 (5) 0.3-2.2	0.6 (7) 0.2-1.2

Cohort 1 (Extra I) was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or through the end of pregnancy (see [Figure 1]), and where the pregnancy resulted in a livebirth or stillbirth.

Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); MPA: mycophenolic acid; Tac: tacrolimus group

Table 12 Prevalence (%) of Major and Minor Malformations Among Livebirths, Stillbirths and Therapeutic Abortions (Terminations) ≥ 12 Weeks for Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 1 [Extra II], Objective 1)

Malformation	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 194	Alt n = 35	Tac n = 107	Alt n = 18	Tac n = 301	Alt n = 53	Tac n = 326	Alt n = 1126	Tac n = 207	Alt n = 179	Tac n = 533	Alt n = 1305
% Major (n) 95% CI	1.5 (3) 0.3-4.5	2.9 (1) 0.1-14.9	3.7 (4) 1.0-9.3	0 (0) 0-18.5	2.3 (7) 0.9-4.7	1.9 (1) 0.0-10.1	4.9 (16) 2.8-7.8	3.6 (40) 2.5-4.8	5.8 (12) 3.0-9.9	2.8 (5) 0.9-6.4	5.3 (28) 3.5-7.5	3.4 (45) 2.5-4.6
% Minor (n) 95% CI	3.6 (7) 1.5-7.3	0 (0) 0-10.0	4.7 (5) 1.5-10.6	0 (0) 0-18.5	4.0 (12) 2.1-6.9	0 (0) 0-6.7	0.9 (3) 0.2-2.7	0.6 (7) 0.3-1.3	1.0 (2) 0.1-3.4	0 (0) 0-2.0	0.9 (5) 0.3-2.2	0.5 (7) 0.2-1.1

Cohort 1 (Extra II) was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or through the end of pregnancy (see [Figure 1]), and where the pregnancy resulted in a livebirth, stillbirth or termination ≥ 12 weeks.

Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); MPA: mycophenolic acid; Tac: tacrolimus group.

Table 13 Prevalence (%) of Major and Minor Malformations Among Livebirths, Stillbirths and Therapeutic Abortions (Terminations) ≥ 12 Weeks for Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases, and Age (< 35, ≥ 35) (Cohort 1 [Extra II], Objective 1)

Malformation	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
< 35 years	**Tac n = 134**	**Alt n = 23**	**Tac n = 85**	**Alt n = 14**	**Tac n = 219**	**Alt n = 37**	**Tac n = 258**	**Alt n = 972**	**Tac n = 178**	**Alt n = 163**	**Tac n = 436**	**Alt n = 1135**
% Major (n)	2.2 (3)	0 (0)	4.7 (4)	0 (0)	3.2 (7)	0 (0)	5.0 (13)	3.1 (30)	5.1 (9)	2.5 (4)	5.1 (22)	3.0 (34)
95% CI	0.5-6.4	0.0-14.8	1.3-11.6	0.0-23.2	1.3-6.5	0.0-9.5	2.7-8.5	2.1-4.4	2.3-9.4	0.7-6.2	3.2-7.5	2.1-4.2
% Minor (n)	3.7 (5)	0 (0)	4.7 (4)	0 (0)	4.1 (9)	0 (0)	1.2 (3)	0.6 (6)	1.1 (2)	0 (0)	1.2 (5)	0.5 (6)
95% CI	1.2-8.5	0.0-14.8	1.3-11.6	0.0-23.2	1.9-7.7	0.0-9.5	0.2-3.4	0.2-1.3	0.1-4.0	0.0-2.2	0.4-2.7	0.2-1.1
≥ 35 years	**Tac n = 60**	**Alt n = 12**	**Tac n = 22**	**Alt n = 4**	**Tac n = 82**	**Alt n = 16**	**Tac n = 68**	**Alt n = 154**	**Tac n = 29**	**Alt n = 16**	**Tac n = 97**	**Alt n = 170**
% Major (n)	0 (0)	8.3 (1)	0 (0)	0 (0)	0 (0)	6.3 (1)	4.4 (3)	6.5 (10)	10.3 (3)	6.3 (1)	6.2 (6)	6.5 (11)
95% CI	0.0-6.0	0.2-38.5	0.0-15.4	0.0-60.2	0.0-4.4	0.2-30.2	0.9-12.4	3.2-11.6	2.2-27.4	0.2-30.2	2.3-13.0	3.3-11.3
% Minor (n)	3.3 (2)	0 (0)	4.6 (1)	0 (0)	3.7 (3)	0 (0)	0 (0)	0.7 (1)	0 (0)	0 (0)	0 (0)	0.6 (1)
95% CI	0.4-11.5	0.0-26.5	0.1-22.8	0.0-60.2	0.8-10.3	0.0-20.6	0.0-5.3	0.0-3.6	0.0-11.9	0.0-20.6	0.0-3.7	0.0-3.2

Cohort 1 (Extra II) was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or through the end of pregnancy (see [Figure 1]), and where the pregnancy resulted in a livebirth, stillbirth or termination ≥ 12 weeks.

Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); MPA: mycophenolic acid; Tac: tacrolimus group.

10.3 Objective 3

10.3.1 Descriptive Data for Objective 3

Objective 3: To estimate prevalence of both spontaneous abortions and stillbirths in female transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.

[Table 14] summarizes the number of female recipients, pregnancies, and pregnancy outcomes for cohort 2. This illustrates the extent to which transplant recipients may be associated with multiple pregnancies, and each pregnancy may have multiple outcomes.

[Table 15] displays characteristics of female transplant recipients, with no exposure to MPA from 6 weeks before conception to up to at least 22 weeks of gestation, and who had any pregnancy outcome (cohort 2). No women initiated MPA after 22 weeks of gestation. The number of women was slightly larger than that presented in [Table 8], and the results were similar to those presented in [Table 8].

Table 14 Summary of Number of Transplant Recipients, Pregnancies and Pregnancy Outcomes for Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 2, Objective 3)

	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac	Alt	Tac	Alt	Tac	Alt	Tac	Alt	Tac	Alt	Tac	Alt
Recipients (n)	174	29	93	16	267	45	277	722	157	113	434	835
Pregnancies	205	33	120	19	325	52	435	1272	274	212	709	1484
Outcomes	213	37	122	19	335	56	447	1323	287	215	734	1538
Livebirths	191	35	106	18	297	53	311	1034	198	163	509	1197
Spontaneous abortions (miscarriage)	18	2	15	1	33	3	116	187	76	34	192	221
Therapeutic abortions (terminations)	2	0	1	0	3	0	9	62	5	13	14	75
Stillbirths	1	0	0	0	1	0	6	30	4	3	10	33
Ectopic	1	0	0	0	1	0	5	10	4	2	9	12
Sets of twins (triplets) [quadruplets]	8	4	2	0	10	4	10 (1)	36 (6) [1]	13	3	23 (1)	39 (6) [1]

Alt: alternative immunosuppressant group; Tac: tacrolimus group.

Table 15 Characteristics of Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 2, Objective 3)

	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 205	Alt n = 33	Tac n = 120	Alt n = 19	Tac n = 325	Alt n = 52	Tac n = 435	Alt n = 1272	Tac n = 274	Alt n = 212	Tac n = 709	Alt n = 1484
Mean (SD) age (years) at first transplant, range	24.6 (6.8) 2.9-40.2	20.8 (6.5) 7.6-34.4	19.2 (10.8) 0.3-40.6	6.1 (7.4) 0.5-27.4	22.6 (8.9) 0.3-40.6	15.4 (9.8) 0.5-34.4	24.8 (6.0) 2.9-44.2	23.1 (6.1) 1.2-42.4	20.8 (8.9) 0.5-42.2	19.5 (7.9) 0.5-37.8	23.2 (7.5) 0.5-44.2	22.6 (6.5) 0.5-42.4
Mean (SD) age (years) at conception, range	32.9 (4.3) 19.2-45.2	33.6 (4.7) 24.7-44.3	31.0 (5.5) 15.9-43.6	29.6 (5.4) 20.1-40.1	32.2 (4.8) 15.9-45.2	32.1 (5.3) 20.1-44.3	31.7 (4.8) 18.9-47.9	29.3 (5.4) 16.4-48.2	28.9 (6.0) 13.2-43.4	28.0 (5.1) 16.3-43.1	30.6 (5.5) 13.2-47.9	29.1 (5.4) 16.3-48.2
Mean (SD) interval (years) from transplant to conception, range	6.7 (4.7) 0.2-22.9	12.1 (7.1) 1.7-27.6	11.9 (8.5) 0.8-35.8	23.5 (4.6) 12.7-30.9	8.6 (6.9) 0.2-35.8	16.3 (8.3) 1.7-30.9	5.5 (3.6) 0.3-18.0	5.4 (4.2) 0.1-27.3	7.9 (6.5) 0.2-27.9	8.4 (7.4) 0.2-30.9	6.4 (5.1) 0.2-27.9	5.8 (4.9) 0.1-30.9
% Pre-transplant pregnancies (n)	29.9 (61)	21.2 (7)	16.7 (20)	0 (0)	25.0 (81)	13.5 (7)	26.9 (117)	23.6 (300)	24.1 (66)	20.8 (44)	25.8 (183)	23.2 (344)

Cohort 2 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or at any time up to 22 weeks of gestation (see [Figure 2]), and where the pregnancy resulted in any pregnancy outcome.

Alt: alternative immunosuppressant group; MPA: mycophenolic acid; Tac: tacrolimus group.

10.3.2 Outcome Data for Objective 3

See [Section 10.3.3] Main Results for Objective 3.

10.3.3 Main Results for Objective 3

[Table 16] utilizes the cohort presented in [Table 15] (i.e., cohort 2), and shows results for prevalence of spontaneous abortions (miscarriages) and stillbirths. Among retrospective cases, prevalence of spontaneous abortions was higher among users of tacrolimus (26.2%, 95% CI [exact Clopper Pearson]: 23.0-29.5) than users of alternative treatments (14.4%, 95% CI: 12.7-16.2). The pattern was similar among prospective cases although prevalence tended to be lower and numbers smaller, rendering estimates more imprecise (9.9%, 95% CI: 6.9-13.6, for tacrolimus; 5.4%, 95% CI: 1.1-14.9, for alternative treatments). There was no suggestion of differences in prevalence between women with kidney or liver transplants.

Among retrospective cases, prevalence of stillbirths was 1.4% for tacrolimus and 2.2% for alternative immunosuppressants with no strong evidence of differences between strata. There was only 1 stillbirth reported among the prospective cases.

Table 16 Prevalence (%) of Spontaneous Abortions and Stillbirths in Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 2, Objective 3)

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 213	Alt n = 37	Tac n = 122	Alt n = 19	Tac n = 335	Alt n = 56	Tac n = 447	Alt n = 1323	Tac n = 287	Alt n = 215	Tac n = 734	Alt n = 1538
% Spontaneous abortion (n) 95% CI	8.5 (18) 5.1-13.0	5.4 (2) 0.7-18.2	12.3 (15) 7.0-19.5	5.3 (1) 0.1-26.0	9.9 (33) 6.9-13.6	5.4 (3) 1.1-14.9	26.0 (116) 22.0-30.3	14.1 (187) 12.3-16.1	26.5 (76) 21.5-32.0	15.8 (34) 11.2-21.4	26.2 (192) 23.0-29.5	14.4 (221) 12.7-16.2
% Stillbirths (n) 95% CI	0.5 (1) 0.0-2.6	0 (0) 0-9.5	0 (0) 0-3.0	0 (0) 0-17.6	0.3 (1) 0.0-1.7	0 (0) 0-6.4	1.3 (6) 0.5-2.9	2.3 (30) 1.5-3.2	1.4 (4) 0.4-3.5	1.4 (3) 0.3-4.0	1.4 (10) 0.7-2.5	2.2 (33) 1.5-3.0

Cohort 2 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the period from 6 weeks prior to conception until the end of the first trimester without use of MPA during this period or at any time up to 22 weeks of gestation (see [Figure 2]), and where the pregnancy resulted in any pregnancy outcome.

Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); MPA: mycophenolic acid; Tac: tacrolimus group.

10.3.4 Additional Results for Objective 3

After adjusting for the range of explanatory variables in the logistic regression analyses as specified in [Section 9.10.4], for prospective and retrospective cases combined, tacrolimus remained associated with spontaneous abortions in the combined kidney and liver group (OR1.70, 95% CI:1.31-2.21). Advanced maternal age (≥ 35 years, compared with < 35 years) was retained in the final model (OR 1.55, 95% CI: 1.02-2.36). After adjusting for the explanatory variables in the logistic regression analyses, there were no variables found to be associated with the risk of stillbirth in analyses in the kidney, liver, or combined kidney and liver group.

10.4 Objective 4

10.4.1 Descriptive Data for Objective 4

Objective 4: To estimate prevalence of small for gestational age (SGA)/intrauterine growth restriction (IUGR) following exposure to tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.

[Table 17] differs from [Table 8] and [Table 15] in summarizing characteristics from cohort 3, i.e., female transplant recipients not excluding those with MPA exposure. Like [Table 8], only pregnancies with livebirth outcomes were included in [Table 17]. Comparing results with those from [Table 8] and [Table 15], there were only minor differences.

Table 17 Characteristics of Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants During Pregnancy by Prospective and Retrospective Cases (Cohort 3, Objective 4)

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 224	Alt n = 37	Tac n = 117	Alt n = 18	Tac n = 341	Alt n = 55	Tac n = 386	Alt n = 1017	Tac n = 210	Alt n = 163	Tac n = 596	Alt n = 1180
Mean (SD) age (years) at first transplant, range	24.6 (6.5) 2.9-40.2	20.8 (6.3) 7.6-34.4	19.7 (10.7) 0.3-40.6	6.0 (7.5) 0.5-27.4	22.9 (8.5) 0.3-40.6	15.9 (9.7) 0.5-34.4	24.4 (6.0) 2.9-44.2	23.0 (6.0) 1.2-42.4	20.1 (8.7) 0.2-42.2	20.1 (7.5) 1.0-37.8	22.9 (7.4) 0.2-44.2	22.6 (6.3) 1.0-42.4
Mean (SD) age (years) at conception, range	32.4 (4.5) 14.5-45.2	33.2 (4.6) 24.7-44.3	31.1 (5.4) 15.9-43.6	29.9 (5.4) 20.1-40.1	31.9 (4.8) 14.5-45.2	32.1 (5.1) 20.1-44.3	30.6 (4.8) 18.9-47.9	29.3 (5.2) 16.4-48.2	28.3 (5.7) 13.2-42.6	28.3 (5.1) 16.3-39.5	29.8 (5.2) 13.2-47.9	29.1 (5.2) 16.3-48.2
Mean (SD) interval (years) from transplant to conception, range	6.2 (4.7) 0.2-22.9	11.8 (6.9) 1.7-27.6	11.2 (8.7) 0.8-35.8	23.9 (4.3) 12.7-30.9	7.9 (6.8) 0.2-35.8	15.8 (8.4) 1.7-30.9	5.0 (3.4) 0.3-19.1	5.5 (4.1) 0.1-22.5	8.0 (6.8) 0.2-27.9	8.2 (6.9) 0.2-30.7	6.0 (5.1) 0.2-27.9	5.8 (4.7) 0.1-30.7
% Pre-transplant pregnancies (n)	30.4 (68)	24.3 (9)	19.7 (23)	0 (0)	26.7 (91)	16.4 (9)	27.2 (105)	24.1 (245)	24.3 (51)	19.6 (32)	26.2 (156)	23.5 (277)

Cohort 3 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy (see [Figure 3]), and where the pregnancy resulted in a livebirth.

Alt: alternative immunosuppressant group; MPA: mycophenolic acid; Tac: tacrolimus group.

10.4.2 Outcome Data for Objective 4

See [Section 10.4.3] Main Results for Objective 4.

10.4.3 Main Results for Objective 4

[Table 18] shows results from use of cohort 3, where there was no exclusion for those with MPA exposure and inclusion of only livebirth outcomes. The characteristics of cohort 3 are described in [Table 17]. Results show pregnancy outcomes for mean gestational age (GA), prematurity (GA < 37 weeks), early pre-term (GA < 34 weeks), mean birth weight, low birth weight (< 2500 g), very low birth weight (< 1500 g), and small for gestational age/intrauterine growth retardation (SGA/IUGR). Mean GA was found to be approximately 1 week shorter among kidney recipients than liver recipients for retrospective cases and approximately 2 weeks shorter for prospective cases. Mean birth weight followed the same pattern, with newborns among kidney recipients on average 100 g to 500 g smaller, with a tendency for bigger differences among prospective cases. There was no evidence of differences in mean birth weight between tacrolimus and alternative treatments. Regarding prematurity, early preterm birth, low birth weight and very low birth weight, they all tended to be more prevalent among kidney recipients than liver recipients, with the difference tending to be greater among prospective cases. There was also a suggestion of a higher prevalence of prematurity and early pre-term birth among tacrolimus users than among those on alternative treatments, and larger proportions of tacrolimus users with low birth weight and very low birth weight. For SGA/IUGR, the main outcome for objective 4, there was a tendency for a lower prevalence in the tacrolimus group than the alternative treatment group.

Table 18 Prevalence (%) of Outcomes at Birth in Liveborn Children of Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 3, Objective 4)

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 233	Alt n = 41	Tac n = 119	Alt n = 18	Tac n = 352	Alt n = 59	Tac n = 395	Alt n = 1059	Tac n = 217	Alt n = 166	Tac n = 612	Alt n = 1225
Mean gestational age (weeks)	35.6	35.9	37.7	38.1	36.3	36.5	35.2	36.0	36.2	37.0	35.6	36.2
Min	23.0	29.0	31.0	35.0	23.0	29.0	21.0	23.0	22.0	26.0	21.0	23.0
Max	40.7	39.9	41.0	42.0	41.0	42.0	42.0	43.0	42.0	42.5	42.0	43.0
Median	36.4	37.0	38.0	38.0	37.0	37.0	36.0	37.0	37.0	38.0	36.5	37.0
SD	3.1	2.9	2.3	2.0	3.0	2.8	3.5	3.4`	3.6	3.1	3.6	3.4
% Premature (< 37 weeks)	55.4	48.8	22.7	27.8	44.3	42.4	57.5	49.6	45.2	35.5	53.1	47.7
(n)	(129)	(20)	(27)	(5)	(156)	(25)	(227)	(525)	(98)	(59)	(325)	(584)
95% CI	48.7-61.9	32.9-64.9	15.5-31.3	9.7-53.5	39.1-49.7	29.6-55.9	52.4-62.4	46.5-52.6	38.4-52.0	28.3-43.3	49.1-57.1	44.8-50.5
% Early preterm (< 34 weeks)	20.6	19.5	9.2	0	16.8	13.6	26.3	19.6	20.3	10.2	24.2	18.4
(n)	(48)	(8)	(11)	(0)	(59)	(8)	(104)	(208)	(44)	(17)	(148)	(225)
95% CI	15.6-26.4	8.8-34.9	4.7-15.9	0-18.5	13.0-21.1	6.0-25.0	22.1-31.0	17.3-22.2	15.1-26.2	6.1-15.9	20.8-27.8	16.2-20.7
Mean birth weight (g)	2566.9	2376.8	2917.4	3028.5	2685.1	2575.6	2468.7	2591.7	2720.0	2748.7	2557.7	2612.7
Min	453.6	1048.9	1400.5	2239.6	453.6	1048.9	397.0	453.6	567.0	680.4	397.0	453.6
Max	4167.4	3339.6	4025.6	3827.2	4167.4	3827.2	4252.4	4536.0	4535 9	4961.2	4535.9	4961.2
Median	2705.0	2494.8	2976.9	2962.6	2806.6	2608.2	2579.8	2714.5	2849.0	2891.7	2693.2	2735.0
SD	752.3	638.7	599.2	429.9	722.9	653.5	791.7	756.4	830.4	709.1	813.9	751.9
% Low birth weight (< 2500 g)	40.8	51.2	21.9	11.1	34.4	39.0	46.6	40.8	34.1	27.7	42.2	39.0
(n)	(95)	(21)	(26)	(2)	(121)	(23)	(184)	(432)	(74)	(46)	(258)	(478)
95% CI	34.4-47.4	35.1-67.1	14.8-30.4	1.4-34.7	29.4-39.6	26.5-52.6	41.6-51.6	37.8-43.8	27.8-40.8	21.1-35.2	38.2-46.2	36.3-41.8
% Very low birth weight (< 1500 g)	8.2	14.6	1.7	0	6.0	10.2	12.4	9.8	9.7	5.4	11.4	9.2
(n)	(19)	(6)	(2)	(0)	(21)	(6)	(49)	(104)	(21)	(9)	(70)	(113)
95% CI	5.0-12.4	5.6-29.2	0.2-5.9	0-18.5	3.7-9.0	3.8-20.8	9.3-16.1	8.1-11.8	6.1-14.4	2.5-10.0	9.0-14.2	7.7-11.0
% SGA/IUGR	17.6	41.5	18.5	11.1	17.9	32.2	19.8	23.3	15.2	24.7	18.1	23.5
(n)	(41)	(17)	(22)	(2)	(63)	(19)	(78)	(247)	(33)	(41)	(111)	(288)
95% CI	12.9-23.1	26.3-57.9	12.0-26.6	1.4-34.7	14.0-22.3	20.6-45.6	15.9-24.0	20.8-26.0	10.7-20.7	18.3-32.0	15.2-21.4	21.2-26.0

Cohort 3 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy (see [Figure 3]), and where the pregnancy resulted in a livebirth.
Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); max: maximum; min: minimum; MPA: mycophenolic acid; SGA/IUGR: small for gestational age/intrauterine growth restriction; Tac: tacrolimus group.

10.4.4 Additional Results for Objective 4

Logistic regression analysis was only conducted for the main outcome for objective 4, i.e., SGA/IUGR. Consistent with [Table 18], there was a decreased risk of SGA/IUGR among tacrolimus users in the combined kidney and liver group (OR 0.70, 95% CI: 0.57-0.87, n = 2248). No other explanatory variables were retained in the model.

10.5 Objective 5

10.5.1 Descriptive Data for Objective 5

Objective 5: To describe the distribution of adverse events in children who were breastfed while their mothers were using tacrolimus (or alternative immunosuppressants, as a combined group).

[Table 19] provides descriptive data on women who breastfed, including mean age at conception and mean duration of breastfeeding. None of the women reported taking MPA while breastfeeding. There were 233 women who reported breastfeeding among prospective cases (203 using tacrolimus, 30 using alternative treatments) and 308 among retrospective cases (193 using tacrolimus, 115 using alternative treatments). There may be a suggestion of tacrolimus users breastfeeding for slightly longer than those on alternative treatments but numbers were small.

Table 19 Characteristics of Female Kidney and Liver Transplant Recipients Who Breastfed Their Children While Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 4, Objective 5)

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 127	Alt n = 23	Tac n = 76	Alt n = 7	Tac n = 203	Alt n = 30	Tac n = 127	Alt n = 100	Tac n = 66	Alt n = 15	Tac n = 193	Alt n = 115
Mean (SD) age (years) at conception, range	32.7 (4.4) 19.2-45.2	33.7 (3.9) 27.9-44.3	30.6 (4.9) 15.9-42.7	27.4 (3.3) 22.4-32.0	31.9 (4.7) 15.9-45.2	32.2 (4.6) 22.4-44.3	31.6 (4.6) 21.3-47.9	32.7 (4.6) 21.2-45.1	29.1 (5.2) 18.3-41.6	30.0 (4.2) 20.1-37.4	30.7 (5.0) 18.3-47.9	32.3 (4.7) 20.1-45.1
Mean (SD) duration (months) of breastfeeding, range	6.3 (5.9) 0.2-33.4	4.0 (3.4) 0.5-12.0	8.3 (8.2) 0.6-35.9	2.2 (1.1) 1.4-3.0	6.9 (6.8) 0.2-35.9	3.9 (3.3) 0.5-12.0	8.9 (9.7) 0.1-47.9	7.6 (8.7) 0.2-41.9	6.8 (7.6) 0.1-44.3	2.8 (3.0) 0.2-8.9	8.2 (9.1) 0.1-47.9	6.9 (8.3) 0.2-41.9

Cohort 4 was defined as breastfed children born to female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during breastfeeding (see [Figure 4]).

Alt: alternative immunosuppressant group; Tac: tacrolimus group.

10.5.2 Outcome Data for Objective 5

See [Section 10.5.3] Main Results for Objective 5.

10.5.3 Main Results for Objective 5

There were no systematic outcomes reported in breastfed children born to female transplant recipients. Prevalence of outcomes in breastfed children were not available from the TPRI.

10.5.4 Additional Results for Objective 5

Of the offspring that were breastfed, 21 recipients reported that tacrolimus levels were tested in the infants and none reported high levels of tacrolimus in the infants' blood or having been advised to stop breastfeeding due to any increase in infections or problems in the infants (data not presented). No mothers reported any adverse events due to breastfeeding.

Of the 145 infants that were breastfed while their mothers were on alternative immunosuppressants, seven mothers reported having their infants tested for cyclosporine levels and one reported that she was advised to stop breastfeeding after 2 weeks since cyclosporine was detected (amount not specified). At the last follow-up, at age 22 years, the child (now an adult) was reported healthy.

10.6 Objective 6

10.6.1 Descriptive Data for Objective 6

Objective 6: To estimate prevalence of major malformations among children whose reported biological fathers were transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group). Similarly, for minor malformations (as a combined group).

[Table 20]draws from cohort 5 and shows characteristics of male recipients who fathered children, and had no MPA exposure around the time of conception. Descriptive statistics were provided for mean age at first transplant, mean age at conception, and mean interval between transplant and conception. There were 5 prospective cases and 800 retrospective (701 kidney, 99 liver; 73 tacrolimus, 727 alternative treatments). Among retrospective cases, mean age at first transplant ranged from 26.8 to 31.9 years, mean age at conception ranged from 31.8 to 36.3 years, and mean interval from transplant to conception ranged from 4.3 to 5.5 years. There were no discernable trends.

Table 20 Characteristics of Male Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 5, Objective 6)

	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 1	Alt n = 0	Tac n = 4	Alt n = 0	Tac n = 5	Alt n = 0	Tac n = 32	Alt n = 669	Tac n = 41	Alt n = 58	Tac n = 73	Alt n = 727
Mean (SD) age (years) at first transplant, range	23.7 (–) 23.7-23.7	N/A	22.6 (5.0) 16.4-27.5	N/A	22.8 (4.3) 16.4-27.5	N/A	27.5 (7.9) 0.2-38.0	26.8 (7.3) 5.7-54.6	31.9 (7.4) 21.0-56.4	30.2 (7.8) 11.8-46.2	30.0 (7.9) 0.2-56.4	27.0 (7.4) 5.7-54.6
Mean (SD) age (years) at conception, range	33.0 (–) 33.0-33.0	N/A	30.5 (2.0) 27.9-32.3	N/A	31.0 (2.1) 27.9-33.0	N/A	34.6 (5.2) 24.8-42.5	31.8 (6.3) 16.2-58.5	36.3 (7.4) 24.3-61.9	34.0 (6.9) 17.8-47.3	35.6 (6.5) 24.3-61.9	31.9 (6.4) 16.2-58.5
Mean (SD) interval (years) from transplant to conception, range	9.3 (–) 9.3-9.3	N/A	5.4 (4.6) 1.3-11.5	N/A	6.2 (4.4) 1.3-11.5	N/A	5.5 (3.8) 1.0-13.7	4.4 (4.4) 0.1-27.8	4.4 (3.3) 0.3-12.6	4.3 (6.6) 0.0-44.0	4.9 (3.5) 0.3-13.7	4.4 (4.7) 0.0-44.0

Cohort 5 was defined as reported pregnancies associated with biological fathers who were kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the 6-week period prior to conception, without use of MPA during the 90-day period prior to conception (see [Figure 5]), and where the pregnancy resulted in a livebirth.

Alt: alternative immunosuppressant group; MPA: mycophenolic acid; N/A: not applicable; Tac: tacrolimus group.

10.6.2 Outcome Data for Objective 6

See [Section 10.6.3] Main Results for Objective 6.

10.6.3 Main Results for Objective 6

Results in [Table 21] were based on cohort 5 and show the prevalence of major and minor malformations in the offspring of female partners of male transplant recipients. Among prospective cases, numbers were too small to make any inferences (5 livebirths). Among retrospective cases, the prevalence of major malformations was 6.3% in the tacrolimus group (95% CI: 2.1-14.0) and 2.9% in the alternative treatment group (95% CI: 1.8-4.3). Numbers were small in the tacrolimus group and hence the lack of precision in the estimate. For minor malformations, the prevalence was 1.3% (1 of 80; 95% CI: 0.0-6.8) among tacrolimus users, and 0.3% (2 of 738; 95% CI: 0.0-1.0) among those on alternative treatments.

10.6.4 Additional Results for Objective 6

The detailed distribution of major and minor malformations combined, among children whose reported biological fathers were kidney or liver transplant recipients using tacrolimus (or alternative immunosuppressants, as a combined group), is presented in [Table 22]. No logistic regression analyses were undertaken for this objective as number of tacrolimus users was relatively small.

Table 21 Prevalence (%) of Major and Minor Malformations Among Livebirth Children Whose Reported Biological Fathers Were Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective And Retrospective Cases (Cohort 5, Objective 6)

Malformation	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 1	Alt n = 0	Tac n = 4	Alt n = 0	Tac n = 5	Alt n = 0	Tac n = 35	Alt n = 678	Tac n = 45	Alt n = 60	Tac n = 80	Alt n = 738
% Major (n) 95% CI	0 (0) 0-97.5	N/A	25.0 (1) 0.6-80.6	N/A	20.0 (1) 0.5-71.6	N/A	8.6 (3) 1.8-23.1	2.9 (20) 1.8-4.5	4.4 (2) 0.5-15.1	1.7 (1) 0.0-8.9	6.3 (5) 2.1-14.0	2.9 (21) 1.8-4.3
% Minor (n) 95% CI	0 (0) 0-97.5	N/A	0 (0) 0-60.2	N/A	0 (0) 0-52.2	N/A	2.9 (1) 0.1-14.9	0.3 (2) 0.0-1.1	0 (0) 0-7.9	0 (0) 0-6.0	1.3 (1) 0.0-6.8	0.3 (2) 0.0-1.0

Cohort 5 was defined as reported pregnancies associated with biological fathers who were kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the 6-week period prior to conception, without use of MPA during the 90-day period prior to conception (see [Figure 5]), and where the pregnancy resulted in a livebirth.

Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); MPA: mycophenolic acid; N/A: not applicable; Tac: tacrolimus group.

Table 22 Number of Each Type of Malformation (Major and Minor Combined) Among Children Whose Reported Biological Fathers Were Kidney or Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 5, Objective 6)

All Malformations	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 1	Alt n = 0	Tac n = 4	Alt n = 0	Tac n = 5	Alt n = 0	Tac n = 35	Alt n = 678	Tac n = 45	Alt n = 60	Tac n = 80	Alt n = 738
Nervous system												
Neural tube defects	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Anencephalus and similar	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Encephalocele	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Spina bifida	–	N/A	–	N/A	–	N/A	1	–	–	–	1	–
Hydrocephalus	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Severe microcephaly	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Arhinencephaly/ holoprosencephaly	–	N/A	–	N/A	–	N/A	–	1+	–	–	–	1
Eye												
Anophthalmos/ micropthalmos	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Anophthalmos	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Congenital cataract	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Congenital glaucoma	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Ear, face and neck												
Anotia	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Other-deaf	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Other-tongue tie*	–	N/A	–	N/A	–	N/A	–	2	–	–	–	2
Congenital heart defects												
Severe CHD	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Common arterial truncus	–	N/A	–	N/A	–	N/A	–		–	–	–	–
Double outlet right ventricle	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Transposition of great vessels	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Single ventricle	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Ventricular septal defect (VSD)	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Atrial septal defect (ASD)	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–

All Malformations	**Prospective**						**Retrospective**					
	Kidney		**Liver**		**Kidney + Liver**		**Kidney**		**Liver**		**Kidney + Liver**	
	Tac n = 1	**Alt n = 0**	**Tac n = 4**	**Alt n = 0**	**Tac n = 5**	**Alt n = 0**	**Tac n = 35**	**Alt n = 678**	**Tac n = 45**	**Alt n = 60**	**Tac n = 80**	**Alt n = 738**
Atrioventricular septal defect (AVSD)	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Tetralogy of Fallot	–	N/A	–	N/A	–	N/A	–	–	–	1	–	1
Tricuspid atresia and stenosis	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Ebstein's anomaly	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Pulmonary valve stenosis	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Pulmonary valve atresia	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Aortic valve atresia/stenosis	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Mitral valve anomalies	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Hypoplastic left heart	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Hypoplastic right heart	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Coarctation of aorta	–	N/A	1	N/A	1	N/A	–	–	–	–	–	–
Aortic atresia/interrupted aortic arch	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Total anomalous pulmonary venous return	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
PDA as only CHD in term infants (≥ 37 weeks)	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Other-coronary artery fistula	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Respiratory												
Choanal atresia	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Cystic adenomatous malformation of lung	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Oro-facial clefts							–					
Cleft lip with or without palate	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Cleft palate	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Digestive system												
Oesophageal atresia with or without tracheo-oesophageal fistula	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Duodenal atresia or stenosis (pyloric stenosis)	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Atresia or stenosis of other parts of small intestine	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Ano-rectal atresia and stenosis	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–

All Malformations	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 1	Alt n = 0	Tac n = 4	Alt n = 0	Tac n = 5	Alt n = 0	Tac n = 35	Alt n = 678	Tac n = 45	Alt n = 60	Tac n = 80	Alt n = 738
Hirschsprung's disease	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Atresia of bile ducts	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Annular pancreas	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Diaphragmatic hernia	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Abdominal wall defects												
Gastroschisis	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Omphalocele	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Urinary												
Bilateral renal agenesis including Potter syndrome	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Multicystic renal dysplasia	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Congenital hydronephrosis	–	N/A	–	N/A	–	N/A	–	–	1	–	1	–
Bladder exstrophy and/or epispadia	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Posterior urethral valve and/or prune belly	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Other-small kidney (dysplastic)	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Other-renal/ureteral reflux	–	N/A	–	N/A	–	N/A	2	2	–	–	2	2
Other-unilateral agenesis of kidney	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Genital												
Hypospadias	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Indeterminate sex	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Others-undescended testicle	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Other-micropenis	–	N/A	–	N/A	–	N/A	1	–	–	–	1	–
Limb												
Limb reduction defects-radial hemimelia b/l 1st metacarpal	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Club foot - talipes equinovarus	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Hip dislocation and/or dysplasia	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Polydactyly	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Syndactyly	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Other anomalies/syndromes												
Skeletal dysplasias	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Craniosynostosis	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–

All Malformations	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 1	Alt n = 0	Tac n = 4	Alt n = 0	Tac n = 5	Alt n = 0	Tac n = 35	Alt n = 678	Tac n = 45	Alt n = 60	Tac n = 80	Alt n = 738
Congenital constriction bands/amniotic band	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
itus inversus*	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Conjoined twins	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Congenital skin disorders*	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Vater/Vacterl	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Vascular disruption anomalies	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Lateral anomalies	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Teratogenic syndromes with malformations	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Fetal alcohol syndrome	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Valproate syndrome	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Maternal infections resulting in malformations	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Genetic syndromes + microdeletion	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Down syndrome	–	N/A	–	N/A	–	N/A	–	1^	–	–	–	1
Patau syndrome/trisomy 13	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Edward syndrome/trisomy 18	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Turner syndrome	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Klinefelter syndrome	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Other-trisomy 4	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Other-Prader-Willi syndrome	–	N/A	–	N/A	–	N/A	–	–	–	–	–	–
Other-osteogenesis imperfecta	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Other-dwarfism	–	N/A	–	N/A	–	N/A	–	1	–	–	–	1
Other-albinism	–	N/A	–	N/A	–	N/A	–	–	1	–	1	–

Cohort 5 was defined as reported pregnancies associated with biological fathers who were kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during the 6-week period prior to conception, without use of MPA during the 90-day period prior to conception (see [Figure 5]), and where the pregnancy resulted in a livebirth.

Alt: alternative immunosuppressant group; ASD: atrial septal defect; AVSD: atrioventricular septal defect; b/l: bilateral; CHD: congenital heart disease; N/A: not applicable; PDA: patent ductus arteriosus; Tac: tacrolimus group; VSD: ventricular septal defect.

+: 1 individual (with arhinencephaly/holoprosencephaly plus b/l cleft lip and palate), recorded as 1 malformation; ^: 1 individual (with down syndrome plus renal reflux), recorded as 1 malformation; * rows with minor malformations

10.7 Objectives 7 & 8

10.7.1 Descriptive Data for Objectives 7 & 8

Objective 7: To estimate prevalence of gestational diabetes mellitus (GDM) following exposure to tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.

Objective 8: To estimate prevalence of gestational hypertension (GH) and pre-eclampsia following exposure to tacrolimus (or alternative immunosuppressants, as a combined group) during pregnancy.

[Table 23] utilizes cohort 6, i.e., not excluding MPA exposures, and including all pregnancy outcomes, and provides results on mean age at first transplant, mean age at conception, mean duration between transplant and conception, and the % of women who experienced a pre-transplant pregnancy. Cohort 6 used the same overall population that was used to generate results in [Table 2]; there were only minor differences in the results compared with [Table 8]. Note that prevalence of GDM was assessed in the sub-set of female recipients of cohort 6 with no evidence of diabetes prior to pregnancy, prevalence of GH was assessed in the sub-set of female recipients of cohort 6 with no evidence of hypertension prior to pregnancy, and prevalence of pre-eclampsia was assessed in the sub-set of female recipients of cohort 6 for whom gestation lasted at least 20 weeks (≥ 140 days).

Table 23 Characteristics of Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 6, Objectives 7 & 8)

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 247	Alt n = 40	Tac n = 136	Alt n = 19	Tac n = 383	Alt n = 59	Tac n = 594	Alt n = 1331	Tac n = 322	Alt n = 216	Tac n = 916	Alt n = 1547
Mean (SD) age (years) at first transplant, range	24.6 (6.7) 2.9-40.2	21.5 (6.6) 7.6-34.8	19.4 (10.4) 0.3-40.6	6.1 (7.4) 0.5-27.4	22.7 (8.6) 0.3-40.6	16.5 (9.9) 0.5-34.8	24.6 (5.8) 2.9-44.2	23.1 (6.1) 1.2-42.4	20.6 (8.8) 0.2-42.2	19.4 (8.0) 0.5-37.8	23.2 (7.3) 0.2-44.2	22.6 (6.5) 0.5-42.4
Mean (SD) age (years) at conception, range	32.5 (4.6) 14.5-47.8	33.2 (4.5) 24.7-44.3	30.8 (5.5) 15.9-43.6	29.6 (5.4) 20.1-40.1	31.9 (5.0) 14.5-47.8	32.1 (5.0) 20.1-44.3	31.0 (4.8) 18.9-47.9	29.3 (5.4) 16.4-48.2	28.7 (5.8) 13.2-43.4	28.0 (5.2) 16.3-43.1	30.2 (5.3) 13.2-47.9	29.2 (5.4) 16.3-48.2
Mean (SD) interval (years) from transplant to conception, range	6.2 (4.6) 0.2-22.9	11.2 (7.0) 1.6-27.6	11.3 (8.4) 0.8-35.8	23.5 (4.6) 12.7-30.9	8.0 (6.7) 0.2-35.8	15.2 (8.6) 1.6-30.9	5.1 (3.5) 0.2-19.1	5.4 (4.1) 0.1-27.3	7.8 (6.5) 0.2-27.9	8.5 (7.4) 0.2-30.9	6.1 (5.0) 0.2-27.9	5.9 (4.9) 0.1-30.9
% of pre-transplant pregnancies (n)	30.8 (76)	27.5 (11)	17.6 (24)	0 (0)	26.1 (100)	18.6 (11)	29.8 (177)	24.6 (328)	23.3 (75)	20.4 (44)	27.5 (252)	24.0 (372)

Cohort 6 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy (see [Figure 6]), and where the pregnancy resulted in any pregnancy outcome.

Alt: alternative immunosuppressant group; MPA: mycophenolic acid; Tac: tacrolimus group.

10.7.2 Outcome Data for Objectives 7 & 8

See [Section 10.7.3] Main Results for Objectives 7 & 8.

10.7.3 Main Results for Objectives 7 & 8

[Table 24] utilizes cohort 6, and presents results on the prevalence of GDM. [Table 25] also utilizes cohort 6, and presents results on prevalence of GH, i.e., new onset hypertension during pregnancy.

Overall, prevalence of GDM was around 7%, with no evidence of a systematic difference across groups. There was an outlier of low prevalence of GDM for liver recipients using alternative treatments among retrospective cases (0.5%, 1 of 210 women).

For new onset hypertension during pregnancy, among retrospective cases, prevalence tended to be higher in the kidney recipients (12.6%, 95% CI: 9.0-17.1 for tacrolimus and 12.1%, 95% CI: 9.8-14.8 for alternative treatments) than the liver recipients (3.5%, 95% CI: 1.6-6.6 for tacrolimus and 6.9%, 95% CI: 3.4-12.3 for alternative treatments). A similar pattern was observed among prospective cases and, overall, prevalence was close to double that seen among retrospective cases, although smaller numbers rendered estimates imprecise.

Prevalence of pre-eclampsia across liver recipient groups was fairly consistent (20.8%-22.2%) (see [Table 26]). The prevalence among kidney recipients on alternative treatments was broadly similar to the risk for liver recipients (18.9% among prospective cases and 25.1% among retrospective cases) but the prevalence among kidney recipients on tacrolimus tended to be higher (37.2% among prospective cases and 34.5% among retrospective cases).

10.7.4 Additional Results for Objectives 7 & 8

Objective 7

In the logistic regression analysis, for BMI prior to pregnancy, each unit increase in BMI was associated with 3% increased risk of GDM in the combined kidney and liver group (OR 1.03, 95% CI 1.00-1.06), and a 7% increased risk of GDM among liver recipients only (OR 1.07, 95% CI 1.01-1.14).

Objective 8

For the logistic regression analysis for GH, neither treatment nor any of the other explanatory variables were retained in the model for the combined kidney and liver group. In the analysis in the liver sub-group, natural conception was retained in the model (OR 2.74, 95% CI: 1.27-5.91).

In addition to results for hypertension presented for new onset hypertension during pregnancy (i.e., GH, see [Table 25]), an additional analysis was undertaken for any hypertension during pregnancy (HTN), which included both new onset hypertension and chronic hypertension (see [Table 27]). For HTN, defined as any hypertension during pregnancy (i.e., including

women with either chronic hypertension or new onset hypertension during pregnancy), and assessed via use of any pharmacologic treatment for hypertension, kidney recipients tended to have higher prevalence of HTN (46.7%-60.0%) than liver recipients (15.8%-31.9%) (see [Table 27]). No consistent pattern was observed between treatment groups.

A logistic regression analysis was undertaken for HTN, defined as any use of hypertensive medication around pregnancy, i.e., including both chronic hypertension and new onset hypertension during pregnancy. Adjusting for explanatory variables (as specified in [Section 9.10]), for the combined kidney and liver group, for each 1-year increase in age at conception, there was a 2% increased risk of HTN, OR 1.02 (95% CI: 1.01-1.03). Tacrolimus use was associated with a decreased risk of HTN in the liver group (OR 0.43, 95% CI: 0.30-0.63), and a trend towards an increased risk of HTN in the kidney group (OR 1.17, 95% CI: 0.99-1.39).

For the logistic regression analysis of pre-eclampsia, only pregnancies with gestation ≥ 20 weeks were included. For prospective and retrospective cases combined, tacrolimus use was associated with an increased risk of pre-eclampsia in the kidney group (OR 1.77, 95% CI: 1.39-2.26), but not in the liver group (OR 0.89, 95% CI: 0.53-1.49). Each unit increase in BMI prior to pregnancy was associated with a 4% increased risk of pre-eclampsia in the liver group (OR 1.04, 95% CI: 1.00-1.09).

Table 24 Prevalence (%) of GDM In Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 6 [GDM], Objective 7)

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 212	Alt n = 37	Tac n = 125	Alt n = 19	Tac n = 337	Alt n = 56	Tac n = 518	Alt n = 1177	Tac n = 273	Alt n = 210	Tac n = 791	Alt n = 1387
% GDM (n), 95% CI	7.5 (16) 4.4-12.0	5.4 (2) 0.7-18.2	4.0 (5) 1.3-9.1	10.5 (2) 1.3-33.1	6.2 (21) 3.9-9.4	7.1 (4) 2.0-17.3	7.9 (41) 5.7-10.6	8.2 (96) 6.7-9.9	4.8 (13) 2.6-8.0	0.5 (1) 0.0-2.6	6.8 (54) 5.2-8.8	7.0 (97) 5.7-8.5

Cohort 6 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy (see [Figure 6]), and where the pregnancy resulted in any pregnancy outcome. For GDM, prevalence was assessed in the sub-set of female recipients of cohort 6 with no evidence of diabetes prior to the pregnancy.

Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); GDM: gestational diabetes mellitus; MPA: mycophenolic acid; Tac: tacrolimus group.

Table 25 Prevalence (%) of GH In Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 6 [GH], Objective 8)

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 145	Alt n = 23	Tac n = 116	Alt n = 17	Tac n = 261	Alt n = 40	Tac n = 277	Alt n = 700	Tac n = 256	Alt n = 145	Tac n = 533	Alt n = 845
% GH (n), 95% CI	20.7 (30) 14.4-28.2	30.4 (7) 13.2-52.9	6.9 (8) 3.0-13.1	5.9 (1) 0.1-28.7	14.6 (38) 10.5-19.4	20.0 (8) 9.1-35.6	12.6 (35) 9.0-17.1	12.1 (85) 9.8-14.8	3.5 (9) 1.6-6.6	6.9 (10) 3.4-12.3	8.3 (44) 6.1-10.9	11.2 (95) 9.2-13.6

Cohort 6 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy (see [Figure 6]), and where the pregnancy resulted in any pregnancy outcome. For GH, prevalence was assessed in the sub-set of female recipients of cohort 6 with no evidence of hypertension prior to the pregnancy,

Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); GH: gestational hypertension; MPA: mycophenolic acid; Tac: tacrolimus group.

Table 26 Prevalence (%) of Pre-eclampsia in Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 6 [Pre-eclampsia], Objective 8)

Parameter	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
	Tac n = 226	Alt n = 37	Tac n = 118	Alt n = 18	Tac n = 344	Alt n = 55	Tac n = 397	Alt n = 1050	Tac n = 216	Alt n = 166	Tac n = 613	Alt n = 1216
% Pre-eclampsia (n), 95% CI	37.2 (84) 30.9-43.8	18.9 (7) 8.0-35.2	22.0 (26) 14.9-30.6	22.2 (4) 6.4-47.6	32.0 (110) 27.1-37.2	20.0 (11) 10.4-33.0	34.5 (137) 29.8-39.4	25.1 (264) 22.5-27.9	20.8 (45) 15.6-26.9	21.7 (36) 15.7-28.7	29.7 (182) 26.1-33.5	24.7 (300) 22.3-27.2

Cohort 6 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy (see [Figure 6]), and where the pregnancy resulted in any pregnancy outcome. For pre-eclampsia, prevalence was assessed in the sub-set of female recipients of cohort 6 for whom gestation lasted at least 20 weeks (≥ 140 days).

Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); MPA: mycophenolic acid; Tac: tacrolimus group.

Table 27 Prevalence (%) of HTN During Pregnancy in Female Kidney and Liver Transplant Recipients Using Tacrolimus or Alternative Immunosuppressants by Prospective and Retrospective Cases (Cohort 6, Objective 8)

	Prospective						Retrospective					
	Kidney		Liver		Kidney + Liver		Kidney		Liver		Kidney + Liver	
Parameter	Tac n = 247	Alt n = 40	Tac n = 136	Alt n = 19	Tac n = 383	Alt n = 59	Tac n = 594	Alt n = 1331	Tac n = 322	Alt n = 216	Tac n = 916	Alt n = 1547
% HTN during pregnancy (n), 95% CI	49.4 (122) 43.0-55.8	60.0 (24) 43.3-75.1	16.2 (22) 10.4-23.5	15.8 (3) 3.4-39.6	37.6 (144) 32.7-42.7	45.8 (27) 32.7-59.2	52.0 (309) 47.9-56.1	46.7 (622) 44.0-49.5	16.5 (53) 12.6-21.0	31.9 (69) 25.8-38.6	39.5 (362) 36.3-42.8	44.7 (691) 42.2-47.2

Cohort 6 was defined as pregnancies among female kidney or liver transplant recipients using tacrolimus-containing regimens (as a combined group, or alternative immunosuppressants, as a combined group) during pregnancy, without restriction on the use of other treatments (e.g., steroids, MPA) before or during pregnancy (see [Figure 6]), and where the pregnancy resulted in any pregnancy outcome.

Alt: alternative immunosuppressant group; CI: confidence interval (exact Clopper Pearson); HTN: hypertension; MPA: mycophenolic acid; Tac: tacrolimus group.

10.8 Adverse Events/Adverse Reactions

As per EMA guidance (see Section VI.C.1.2.1.2 of GVP Module VI), adverse events were not reported in this study using secondary use of data.

11 DISCUSSION

11.1 Key Results

In this study, there were 6 separate cohorts (see [Table 1]) and the presentation of results was stratified by 3 factors: prospectively- or retrospectively-reported cases, kidney or liver transplant recipients, and tacrolimus-containing regimens or non-tacrolimus-containing regimens. Cohorts 1, 2 and 3 are female transplant recipients differing in MPA exposure and pregnancy outcomes. Cohort 1 included females who have no MPA exposure between 6 weeks before conception through to the end of the pregnancy, and have livebirths. Cohort 2 included females with no MPA exposure between 6 weeks before conception through to 22 weeks of gestation, and had any birth outcome. Since there were no recipients in the registry that started MPA after 22 weeks gestation, those who had livebirths in cohort 2 were identical to cohort 1. Cohort 3 did not exclude females with MPA exposure before or during pregnancy, and only included women who had livebirths. As with cohort 3, cohort 4 included all female transplant recipients who delivered a livebirth, but was restricted to those who breastfed their offspring. Cohort 5 included male transplant recipients with no MPA exposure during the 90 days before conception. And cohort 6 included all females including those with MPA exposure before or during pregnancy, irrespective of pregnancy outcomes. The differences in characteristics observed in the 5 female cohorts were generally small, apart from the size of the cohorts and differences in the number of pregnancy outcomes within each cohort.

It would be expected that prospective reporting of pregnancy outcomes, compared to retrospective reporting, may have less reporting bias. Our findings for major malformations showed a tendency for a lower proportion of major malformations among prospective cases, but numbers were relatively limited for the prospective cases. The TPRI began collecting retrospective information in 1991, with pregnancies dating back to 1968, but only began collecting prospective reports in 2010. Thus, on average, the retrospective cases disproportionately reflect older reports to the TPRI. Additionally, some women reporting a livebirth outcome retrospectively were found, upon questioning by the TPRI, to have had previous unreported and unsuccessful (i.e., non-livebirth) post-transplant pregnancies. Hence, the assumption that prospective reporting is subject to more complete and less biased reporting is (at least in part) offset by the additional information gathered on past pregnancies at the time of reporting of retrospective cases.

Regarding year of transplant, the FDA first approved cyclosporine in 1983 (for kidney transplants) and tacrolimus in 1994 (for liver transplants), with a subsequent rapid adoption of tacrolimus as the primary immunosuppressant to prevent liver transplant rejection. For US

kidney recipients, the adoption of tacrolimus was more gradual over the subsequent decade before tacrolimus also became the most used immunosuppressant for kidney transplants.

Objective 1

There was a tendency for lower prevalence of major malformations in the prospective groups than in the retrospective groups. The observed prevalence estimates were similar to the 3% prevalence that has been reported in the general population of the United States [Hoyert et al, 2006; Yoon et al, 1997].

Contrary to the findings for major malformations among offspring of female recipients, there was a trend towards higher prevalence of minor malformations associated with prospective reporting, in particular for tacrolimus. This is consistent with the expectation of a higher proportion of minor malformations being captured among prospective cases than retrospective cases where minor malformations may not be reported so readily. The additional impact of the shift to proportionately more prospective cases among more recent transplants, in light of other changes associated with transplantation, e.g., increasing prevalence of breastfeeding by transplant recipients, is discussed below.

A sensitivity analysis was considered in order to explore the impact of shifting the conception date to 7 days before or 7 days after the estimated conception date, given the uncertainty with regards to conception date. In practice, since only four patients changed their treatment regimen during the study (and none of these was associated with a malformation), formal sensitivity analyses were not undertaken since change in conception date could not have impacted estimates of the prevalence of malformations.

An additional sensitivity analysis was planned to explore the impact, on prevalence of malformations, of including exposure to tacrolimus-containing regimens or alternative immunosuppressants during the first, second or third trimesters of pregnancy (rather than just the first trimester, as per objective 1). Since there were just two recipients who switched from the alternative immunosuppressant group to the tacrolimus-containing regimen group during the second trimester and one during the third trimester, and none of these resulted in a malformation, the overall prevalence of malformations among livebirths in the tacrolimus-containing regimens (combining prospective and retrospective cases, and kidney and liver transplants) was not impacted by this change (3.7%, 30/806 compared with 30/809).

Similarly, given there were no treatment switchers during the 6 weeks prior to conception, a sensitivity analysis associated with adopting a 2-week exposure window prior to conception (rather than a 6-week window) resulted in no change in outcome estimates.

The study captures pregnancies following the emergence of SARS-CoV-2 infection. In the TPRI, over 95% of the deliveries (up to Dec 2020) preceded the emergence of SARS-CoV-2, hence the impact on the study outcomes was expected to be small. To validate this, a sensitivity analysis was conducted excluding the TPRI data after 31 Dec 2019. Prevalence of major malformations did not change markedly by excluding data from 2020. For example,

prevalence among kidney and liver recipients combined for prospective cases for the tacrolimus and alternative immunosuppressant groups were 2.4% (6/246) and 1.9% (1/53) respectively, compared with 2.0% (6/297) and 1.9% (1/53) respectively in the full dataset. Similarly for retrospective cases, prevalence in the tacrolimus and alternative immunosuppressant groups prior to 2020 were 4.8% (24/497) and 3.1% (37/1196) respectively, compared with 4.7% (24/509) and 3.1% (37/1197) in the full dataset.

As per the protocol, a further sensitivity analysis was conducted to investigate prevalence of major malformations in non-tacrolimus-containing cohorts restricted to patients exposed to another calcineurin inhibitor, cyclosporine (+/- prednisone +/- azathioprine), and to patients exposed to prednisone +/- azathioprine (i.e., without a calcineurin inhibitor). The prevalence among retrospectively-reported cases in the cyclosporine group was 3.9% (95% CI: 2.6-5.4, n=31/803), and the prevalence in the prednisone +/- azathioprine group was 1.6% (95% CI: 0.6-3.4, n=6/380). The prevalence in the tacrolimus group was 4.7% (95% CI: 3.0-6.9), i.e., relatively similar to that in the group with the alternative calcineurin inhibitor.

Objective 2

In [Table 10], there is no strong systematic pattern of malformations. There is an outlier of 10 of 350 children among the prospectively-reported recipients (5 among 226 kidney recipients, and 5 among 124 liver recipients) with the minor malformation of tongue tie or ankyloglossia (see ear, face and neck subsection). All 10 children were in the tacrolimus-containing regimen group (3.4%). In comparison, there were 4 ankyloglossia cases among 1706 retrospectively-reported recipients (3 among 1345 kidney recipients, and 1 among 361 liver recipients). Two cases were from the tacrolimus-containing regimen group and 2 from the non-tacrolimus-containing regimen group. This finding may be explained at least in part by a confluence of factors including the relatively recent increase in breastfeeding, the increase in diagnosis of tongue tie, and the proportionate increase in both prospectively-reported cases and tacrolimus users in the TPRI. In more detail: (1) tongue tie is a relatively minor malformation, and is more likely to be captured among prospective than retrospective reports; (2) breastfeeding has increased in transplant recipients over the last decade, associated with the TPRI report in 2013 on the relative safety of breastfeeding for non-MPA using female transplant recipients [Thiagarajan et al, 2013]; (3) tongue tie is more often identified and subsequently reported among women breastfeeding than those bottle feeding, and there has been an increase in referrals to pediatric surgery specialists and provisional diagnoses of tongue tie associated with breastfeeding compared to bottle feeding [Lisonek et al, 2017]; (4) The TPRI started including prospective cases in 2010, and since then there has been an increase in reporting of prospective cases, such that starting in 2015 on average about 40% of pregnancy outcomes reported were prospective; and (5) among prospective cases, 85% (252/295) received tacrolimus, compared with 32% (375/1160) among retrospective cases. For context, in the general population, prevalence of tongue tie

has been reported at between 4.1% and 4.8% [Ricke et al, 2005; Ballard et al, 2002; Messner et al, 2000].

After ear, face and neck, the second most common region for malformations was genital with 2 in the prospective group and 17 in the retrospective group, distributed as 17 in kidney and 2 in liver recipients. Hypospadias was the most common of these malformations, with 12 cases.

There were no cases of microtia, the most prevalent malformation seen in the MPA embryopathy, consistent with the exclusion of MPA in this cohort. Prevalence levels of other components of the MPA embryopathy were also low in this cohort, including oro-facial clefts (n = 5, 0.24%) and congenital heart defects (n = 11, 0.54%).

Objective 3

The prevalence of spontaneous abortions tended to be higher among users of tacrolimus than alternative immunosuppressants, respectively (8.5% and 5.4% in kidney recipients among prospective cases, 12.3% and 5.3% in liver recipients among prospective cases, 26.0% and 14.1% in kidney recipients among retrospective cases, and 26.5% and 15.8% in liver recipients among retrospective cases) (see [Table 16] for respective CIs). For the prospective reports, the prevalence was lower but the numbers smaller, rendering estimates less precise. There was no evidence of differences between kidney and liver transplants. The prevalence of stillbirth in the retrospective reports was 1.4% for tacrolimus and 2.2% for alternative immunosuppressants with no strong evidence of differences between strata. There was only one stillbirth among the prospective cases.

A sensitivity analysis was planned to explore the impact, on prevalence of spontaneous abortions and stillbirths, of including exposure to tacrolimus-containing regimens or alternative immunosuppressants during the first 22 weeks of pregnancy (rather than just the first trimester, which was the period used in objective 3, cohort 2). As noted above, there were only two recipients who switched during the second trimester; they switched from the alternative immunosuppressant group to a tacrolimus-containing regimen. One resulted in a livebirth and one resulted in a spontaneous abortion. The overall prevalence of stillbirths was not impacted by these switchers, and the impact on spontaneous abortions was negligible.

In the 2013 TPRI Annual Report, prevalence of spontaneous abortions was reported for tacrolimus (24.5%, n = 334) and cyclosporine (12.0%, n = 528) users. Although numbers were smaller, prevalence estimates showed the same trend as in the current study.

An additional sensitivity analysis was planned to further explore the impact, on prevalence of stillbirths, of including exposure to tacrolimus-containing regimens or alternative immunosuppressants during the entire pregnancy (rather than just the first trimester, the period used in objective 3, cohort 2). Similar to the previous sensitivity analysis, the overall prevalence of stillbirths was not impacted by this change.

Objective 4

In our study population, mean gestational age was found to be on average 1 to 2 weeks shorter among kidney recipients than liver recipients, with a tendency for a larger difference in prospective cases. Consequently, mean birth weight tended to follow the same pattern, with newborns among kidney recipients on average 100-500 g smaller, with a trend towards larger differences in the prospective cases. As a result, female kidney recipients tended to have higher prevalence of prematurity, early preterm birth, low birth weight and very low birth weight than their liver counterparts (see [Table 18]). Compared to female liver recipients, higher prevalence of prematurity and lower birth weight have been reported for kidney recipients [Deshpande et al, 2012]. Differences in level of kidney function and prevalence of chronic hypertension, both of which favor liver recipients, may (in part) account for these differences.

There was also a suggestion of a higher prevalence of prematurity and early pre-term birth among tacrolimus users than among those on alternative treatments, and consistent with this larger proportions of tacrolimus users with low birth weight and very low birth weight. However, overall, there was an observed decreased risk of SGA/IUGR among tacrolimus users.

Objective 5

In the TPRI, the offspring of female transplant recipients were generally reported to be healthy and developing well. No recipients reported problems in their offspring specific to breastfeeding.

In total, there were 396 breastfed offspring of mothers on tacrolimus, and 145 on alternative immunosuppressants. Breastfeeding increased among female transplant recipients since 2013 following publications by the TPRI [Thiagarajan et al, 2013] and two other groups [Bramham et al, 2013; Zheng et al, 2013] reporting the relative safety of breastfeeding for non-MPA-taking transplant recipients.

Objective 6

With respect to major and minor malformations among livebirth children of fathers with kidney and liver transplants, among prospective cases, numbers were small (5 livebirths) with 1 major malformation. For retrospective cases, the prevalence of major malformations was 6.3% for tacrolimus (95% CI: 2.1-14.0) and 2.9% for the alternative immunosuppressant group (95% CI: 1.8-4.3). There was no strong evidence of a difference given the large and widely overlapping CIs. For minor malformations, the prevalence was 1.3% (1 of 80; 95% CI: 0.0-6.8) among tacrolimus users and 0.3% (2 of 738; 95% CI: 0.0-1.0) among the alternative treatment group.

In general, prospective cases among men were rarely reported, given men tend to be less eager to be interviewed and less forthcoming when interviewed (personal communication, the TPRI).

Despite the interest and enthusiasm of its female participants, in particular, the relatively recent addition of prospective reporting to the TPRI (since 2010) has been linked to a wariness of the TPRI's institutional review board to approve information gathering prior to what they considered the index event, the pregnancy outcome, rather than the pregnancy itself. In practice, males have rarely contacted the TPRI before attempting conception, and rarely before their partner's pregnancy is complete. The small number of prospective reports among males reflects this. However, pregnancy outcomes from male transplant recipients (primarily from retrospective cases) have remained relatively steady over the years.

Objective 7

The prevalence of GDM in the general population varies depending on screening and the prevalence of risk factors, including obesity, and has been reported as 3% to 5% in the UK and up to 40% where obesity is more prevalent [CEMACH, 2005]. In this study population, the prevalence of GDM was around 7%, with no evidence of a systematic difference across groups. There was an outlier for female liver recipients using alternative immunosuppressive treatments among the retrospective cases (0.5%, 1 of 210 women).

The lack of difference in GDM risk associated with use of tacrolimus is not necessarily consistent with reports of diabetogenicity of tacrolimus compared to cyclosporine [Shivaswamy et al, 2016]. However, the finding could for example be explained if more diabetes-prone recipients were already diabetic prior to their pregnancy. Alternatively, the finding would be consistent with healthcare practitioners channeling more diabetes-prone recipients away from use of tacrolimus towards alternative treatment opportunities.

Objective 8

For new onset hypertension during pregnancy (GH), among retrospective cases, prevalence was about 2-fold higher for kidney recipients than liver recipients with no evidence of a substantial difference between tacrolimus and alternative treatments. A similar pattern was observed among prospective cases although the prevalence was approximately double that of retrospective cases.

For hypertension during pregnancy (combining chronic hypertension with GH), the prevalence was about 50% to 60% in kidney recipients, regardless of immunosuppressant or reporting group. The prevalence in liver recipients was lower, i.e., about 15%, except for an outlier in the retrospective alternative immunosuppressant group (32%, 69 of 216 patients). There were mixed results for tacrolimus use from the logistic regression analyses, with a decreased risk of HTN in the liver group (OR 0.43, 95% CI: 0.30-0.63), and a trend towards an increased risk of HTN in the kidney group (OR 1.17, 95% CI: 0.99-1.39).

For pre-eclampsia, higher prevalence has been reported in female kidney recipients compared to liver recipients [TPRI Annual Report, 2016]. The results from this current study were broadly consistent with this. Risk of pre-eclampsia across all liver recipient groups was consistently about 21%. The risk among kidney recipients was generally similar for those on alternative treatments (18.9% among prospective cases and 25.1% among retrospective cases) but higher among those on tacrolimus (37.7% among prospective cases and 34.6% among retrospective cases). In the logistic regression analyses, tacrolimus was associated with an increased risk of pre-eclampsia in the kidney group (OR 1.77, 95% CI: 1.39-2.26), but not the liver group (OR 0.89, 95% CI: 0.53-1.49). The reason for this finding is not clear, but results should be interpreted with caution given substantial changes in diagnostic criteria for pre-eclampsia during the study period.

11.2 Limitations

The results of this study should be considered in light of a number of limitations. Data used in this study were collected from self-reports and interviews which are prone to reporting bias, and missing or incomplete data from the participants.

The sample is also susceptible to selection bias since the registry is entirely voluntary, and thus specific types of participants may be more interested in signing up with the registry introducing limitations around representativeness of the study population.

The retrospective cases have been reported after the pregnancy is completed, and there may be a tendency for either more negative or more positive pregnancy outcomes to be included in the Registry. The prospective cases would be expected to be less biased.

In this study, data collection was only retrospective up until 2010; prospectively-reported cases were also included from 2010. The prospective cases thus tend to have shorter follow-up time. Also, given the shift to proportionately more prospective cases over time, disease management and treatment paradigms may be quite different for prospective and retrospective cases, reflecting the changing practices since the 1990s. Results of sensitivity analyses found that, among retrospectively-reported cases delivered prior to 2010, the prevalence of major malformations among livebirths for tacrolimus users among kidney and liver recipients combined was 6.7% (17/253, 95% CI: 4.0-10.5), and for alternative immunosuppressant users 3.1% (36/1148, 95% CI: 2.2-4.3). Among retrospectively-reported cases delivered since 2010, the prevalence of major malformations among livebirths for tacrolimus users among kidney and liver recipients combined was 2.7% (7/256, 95% CI: 1.1-5.6), and for alternative immunosuppressant users 2.0% (1/49, 95% CI: 0.1-10.9). Given the trend for higher prevalence of major malformations prior to 2010 among tacrolimus users in particular, the analysis of the full dataset may not optimally reflect the situation in recent years. However, restricting the analysis to more recent data would reduce the precision of the estimates in our analysis.

Approximately 93% of the study population was from the United States, and thus the sample may not be broadly generalizable to populations outside the US. Study participants were predominantly Caucasians and lack of ethnic diversity may influence representativeness both in the US and globally.

Sensitized patients have increased rates of both antibody-mediated rejection and acute cellular rejection, as well as higher rates of graft loss, where sensitization is defined by breadth of anti-HLA (Human Leukocyte Antigen) antibodies. There is at least one report of increased risk of poor pregnancy outcomes and accelerated graft loss in a single center sensitized recipient report [Ajaimy et al, 2016]. Relatively few of the TPRI participants may be sensitized (noting that there are variable definitions of "sensitized") and as patients are not consistently aware of their sensitization status, it is not reliable information and hence not routinely obtained by the TPRI. The impact of sensitization status on this analysis is unknown.

Minor malformations appear to be more commonly reported among prospectively collected data. Thus results related to minor malformations from retrospective cases should be interpreted with caution as they are likely to be underrepresented, in particular resulting from under-reporting. On the other hand, from prospective cases, as described earlier, there may be a tendency to over-report certain (transient) minor malformations such as tongue tie, given the relatively recent increase in breastfeeding, the increase in diagnosis of tongue tie, and the proportionate increase in both prospectively-reported cases and tacrolimus users in the TPRI.

The other non-MPA immunosuppressants (azathioprine, cyclosporine, everolimus, prednisone and sirolimus) are not known to be strongly associated with outcomes investigated in this study and have not been evaluated for their impact on the results of the different objectives.

No data were available for analysis of non-immunosuppressant co-medications, and thus it was not possible to include these in the logistic regression analyses to adjust for any potential differences in their distributions.

There was a tendency for the alternative immunosuppressant group to be on average younger at transplantation. This age difference has an unknown impact on pregnancies and outcomes. Age at transplantation was not used as an explanatory variable in the regression models.

As the outcomes of primary interest are related to drug exposure during pregnancy, we opted for pregnancies as the unit of analysis and included all pregnancies, including multiple gestations during a pregnancy and serial pregnancies. While this may introduce bias into analysis of outcomes such as gestational age or pre-eclampsia, it should introduce minimal bias into analysis of malformations. However, since the unit of analysis was pregnancies rather than individuals, it would be appropriate to make an adjustment to the calculations of the confidence intervals to account for those individuals contributing to more than one observation. We have not performed this refinement in the current study. For the different

analyses, the average number of pregnancies per individual ranged between 1 and 2 (see [Table 14] for an example).

The non-tacrolimus-containing regimens' group included 29 pregnancy outcomes among female recipients receiving no immunosuppressants during the study periods. There were 21 livebirths (8 among prospective [5 kidney, 3 liver recipients], 13 among retrospective [5 kidney, 8 liver recipients]), 7 spontaneous abortions (1 among prospective [liver recipient], 6 among retrospective [1 kidney, 5 liver recipients]), 1 stillbirth (retrospective [liver recipient]), and no malformations reported in these 29 outcomes. Had these individuals been excluded from the alternative immunosuppressant group, the prevalence of major malformations would have been similar or slightly higher, e.g., for prospective cases, prevalence of major malformations among livebirths of female kidney and liver recipients would have been 2.2% (1/45) rather than 1.9% (1/53), and for retrospective cases, prevalence of major malformations among livebirths of female kidney and liver recipients would have remained at 3.1%, 37/1184 compared with 37/1197. Given these 29 individuals represent between 1% and 2% of the overall study sample, the impact on the other objectives would also be expected to be small.

With respect to censoring, among prospectively-reported cases, 20 women (4.5%) died or were lost to follow-up, of whom 10 had known treatment regimens, 9 (90%) on tacrolimus-containing regimens, and 1 (10%) on a non-tacrolimus-containing regimen. This closely reflects the proportion of women overall on tacrolimus-containing regimens (87%), and on non-tacrolimus-containing regimens (13%). Of these 20 women, there was 1 recorded maternal death, a kidney recipient who was on an unknown treatment regimen and died before giving birth. There were also 19 women lost to follow-up, 9 on tacrolimus-containing regimens (7 kidney and 2 liver recipients), 1 on a non-tacrolimus-containing regimen (a kidney recipient) and 9 with unknown treatment regimens (6 kidney and 3 liver recipients). When a recipient withdraws consent, their information is deleted from the TPRI. The number of people withdrawing consent are not tracked in the TPRI but, from verbal communication with the TPRI staff, fewer than 10 recipients have withdrawn consent since the start of reporting of prospectively-reported cases (i.e., 2010).

11.3 Interpretation

In this study, there was no evidence of an association of tacrolimus-containing regimens with major malformations. However, the number of events was relatively small, especially in the prospective group, and thus estimates tended to be imprecise. Prevalence of malformations was studied for livebirths in the main analyses. From sensitivity analyses, we found that results were not impacted by including stillbirths and terminations.

Results showed that tacrolimus was associated with increased prevalence of spontaneous abortions (miscarriages). The finding was consistent across retrospectively- and prospectively-reported cases, and across kidney and liver recipients, and remained relatively strong after adjusting for a range of explanatory variables. Although this was a secondary

objective and this descriptive study was not designed to specifically compare across treatment groups, the strength and internal consistency of the evidence generated, together with a previous signal, strengthen the evidence for the association between use of tacrolimus-containing regimens during pregnancy and spontaneous abortions.

Data on hypertension were presented separately for two different definitions of GH: new onset hypertension during pregnancy and any drug-treated hypertension during pregnancy, which included both new onset hypertension and chronic hypertension. Results for tacrolimus were mixed.

Regarding pre-eclampsia, in kidney recipients, there was a trend towards a higher prevalence in the tacrolimus group than in the alternative treatment group. However the reason for this finding is not clear, and results should be interpreted with caution given substantial changes in diagnostic criteria for pre-eclampsia during the study period which may distort the findings.

Numbers were generally relatively small for prospectively-reported cases and thus any inferences drawn need to be made with caution.

11.4 Generalizability

Sampling method and representativeness influence our ability to generalize the results. Voluntary participation and the self-reporting of data collection may make it more difficult to generalize the results of this study to the US population, and more broadly. At the same time, tacrolimus is increasingly becoming a mainstay of immunosuppressive therapy. The shift towards increased use of tacrolimus over time in this Registry may help to make the results of this study more generalizable.

It is known that major malformations affect around 3% of all babies born in the US each year [Hoyert et al, 2006; Yoon et al, 1997]. In this study, the prevalence of major malformation for prospective kidney and liver transplant recipients respectively using tacrolimus was of the order of that reported for the general US population 1.6% (95% CI: 0.3-4.5) and 2.8% (95% CI: 0.6-8.0), and this contrasted with slightly higher prevalence observed in retrospective kidney and liver recipients using tacrolimus 4.8% (95% CI: 2.7-7.8) and 4.6% (95% CI: 2.1-8.5). However, as described above, sensitivity analyses found that prevalence of major malformations tended to be lower after 2010, and more comparable with the estimates from prospective cases.

For minor malformations, prevalence trended higher for prospective kidney and liver transplant recipients respectively using tacrolimus, 3.7% (95% CI: 1.5-7.4) and 4.7% (95% CI: 1.5-10.7), than for retrospective kidney and liver transplant recipients using tacrolimus 1.0% (95% CI: 0.2-2.8) and 1.0% (95% CI: (0.1-3.6). As noted above, estimated prevalence of minor malformations from retrospective cases should be interpreted with caution as they are likely to be under-reported and, certain minor malformations from prospective cases may have a tendency to be over-reported.

Prevalence of spontaneous abortions (miscarriages) tended to be higher among tacrolimus users than among those on alternative immunosuppressants and, among retrospective cases (for tacrolimus: 26.2% 95% CI: 23.0-29.5), at the higher end of the range of what has been reported in more general populations (range: 13.5%-21.6%) [Buss et al, 2006; Nybo Andersen et al, 2000; Armstrong et al, 1992].

Annually, 5% to 9% of pregnancies in the US are affected by GDM [CDC, 2024]. In this study, the prevalence of GDM in both prospective and retrospective kidney and liver transplant recipients fell within this range. In the prospective kidney and liver transplant recipients using tacrolimus, prevalence was 7.5% (95% CI: 4.4-12.0) and 4.0% (95% CI: 1.3-9.1) respectively. In the retrospective kidney and liver transplant recipients using tacrolimus, prevalence was 7.9% (95% CI: 5.7-10.6) and 4.8% (95% CI: 2.6-8.0) respectively.

Hypertensive disorders, including pre-eclampsia and eclampsia, affect 10% of pregnancies in the US [Magley & Hinson, 2023]. In population-based studies using administrative data, the reported US prevalence of hypertensive disorders was 3.0% to 3.8% for pregnancy-induced hypertension and 3.0% to 3.4% for pre-eclampsia [Butwick et al, 2020]. In this TPRI study, the prevalence of pregnancy-induced hypertension in prospective kidney and liver transplant recipients respectively using tacrolimus (12.2% (95% CI: 8.3-16.9) and 5.9% (95% CI: 2.6-11.3) tended to be a little higher than for retrospective cases (5.9% (95% CI: 4.1-8.1) and 2.8% (95% CI: 1.3-5.2) and were of the same order of magnitude as those in the general US population and in population-based studies. The prevalence of pre-eclampsia in this study in both tacrolimus-containing regimens and non-tacrolimus-containing regimens for both kidney and liver recipients was higher than that in the general US population and in published population-based studies, which may largely be study related, e.g., related to study-specific definition of pre-eclampsia.

As noted above in the limitations, approximately 93% of the study population was from the USA, and thus the sample may not be broadly generalizable to populations outside the US. Also study participants were predominantly Caucasian and thus may not be representative of either the US or more globally.

12 OTHER INFORMATION

Not applicable.

13 CONCLUSIONS

In this study of kidney and liver transplant recipients, compared to users of non-tacrolimus-containing regimens, there was no evidence of an association between use of tacrolimus-containing regimens during pregnancy and an increased risk of major or minor malformations. There was however an association between use of tacrolimus-containing regimens during pregnancy and an increased risk of spontaneous abortions. Tacrolimus use during pregnancy tended to be associated with prematurity but not mean birth weight nor

small for gestational age/intrauterine growth restriction. The TPRI did not collect outcomes systematically from children, and so an assessment of the impact of the use of tacrolimus-containing regimens during breastfeeding could not be undertaken. Similarly, pregnancy outcomes associated with male transplant recipients using tacrolimus-containing regimens could not be evaluated since data were limited. There was no strong evidence that use of tacrolimus-containing regimens during pregnancy was associated with gestational diabetes mellitus or gestational hypertension. The prevalence of pre-eclampsia was found to be higher in the sub-group of kidney recipients using tacrolimus during pregnancy than in kidney recipients using alternative treatments or in liver recipients, however, the reason for this finding is not clear, and results should be interpreted with caution especially given lack of consistency in findings across kidney and liver recipients and substantial changes in diagnostic criteria for pre-eclampsia over the study period.

Overall, results from this study need to be considered in the context of study limitations and potential biases including those associated with the voluntary nature of the study, principally retrospectively-reported data, differences between the treatment groups which could not be controlled for, the design of the study being broadly descriptive, and other factors.

However, even given these limitations, it should be noted that this study used data from the largest and most complete registry of transplant patients systematically collecting information on pregnancy outcomes. The results in general should be reassuring to those transplant recipients who wish to become parents, and in particular the results for the primary objective which showed no evidence of tacrolimus-containing regimens (excluding MPA) or non-tacrolimus-containing regimens (excluding MPA) being associated with elevated prevalence of major malformations.

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15 ANNEXES

Annex 1 List of Stand-alone Documents

None.

Signatures

PRINCIPAL INVESTIGATOR'S SIGNATURE

I have read all pages of this final study report for which Astellas is the Sponsor. I confirm that, to the best of my knowledge, it accurately describes the conduct and results of the study.

Principal Investigator: *PPD*

KEY CONTRIBUTORS

The following contributors reviewed this revised final study report (v2.0) with respect to consistency, completeness and traceability of the scientific content, as well as for the accurate representation of the data/information and its interpretation in the document, as relevant to their indicated discipline or role.

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I have read this report and confirm that, to the best of my knowledge, it accurately describes the conduct and results of the study.

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