SYNOPSIS

Name of Sponsor/Company: Rotunda Hospital Name of Finished Product: Tromalyt® Name of Active Ingredient: Acetylsalicylic acid	Individual Study Table Referring to Part of the Dossier Volume: Page:	*(For National Authority Use only)*
Title of Study: **IRELAnD**: Investigating the Role of Early Low-dose Aspirin in Diabetes		
Investigators: Chief Investigator/ Rotunda Principal Investigator – Prof Fionnuala Breathnach NMH Principal Investigator – Prof Mary Higgins Coombe Principal Investigator – Dr Neil O'Gorman Cork Principal Investigator – Dr Mairead O'Riordan Galway Principal Investigator – Dr Geraldine Gaffney Our Lady of Lourdes Principal Investigator – Dr Vineta Ciprike		
Study centre(s): **Multi-site (6 recruitment centres):** Rotunda Hospital - Dublin National Maternity Hospital - Dublin Coombe Women and Infants University Hospital - Dublin Cork University Maternity Hospital - Cork University Hospital Galway - Galway Our Lady of Lourdes Hospital - Drogheda		
Publication (reference): Society for Maternal Fetal Medicine abstract submission ID:1577676 (Abstract Number 26) American Journal of Obstetrics and Gynecology publication reference: AJOGMF_101297		
Studied period (years): 4.5 (date of first enrolment): 23/12/2019 (date of last completed): 21/03/2023	Phase of development: Phase III	

Objectives:

Primary Objective: Investigation of the effect of aspirin therapy initiated in the first trimester of pregnancy in women with pregestational type I or type II diabetes on a composite clinical measure of placental dysfunction (preeclampsia, preterm birth less than 34 weeks, birth weight below the 10th centile or perinatal mortality).

Secondary Objectives:

a. Differences between the intervention and control groups were measured for the following parameters of neonatal morbidity (Gestational age at delivery, Birth weight, NICU admission, Respiratory morbidity, Apgar score <7 at 5 minutes, Umbilical artery acidosis at birth (cord pH <7.2), Interventricular haemorrhage, Culture-proven sepsis, Necrotising enterocolitis, Hypoxic ischaemic encephalopathy).

b. Differences between the aspirin and control groups were measured for maternal outcomes not directly related to primary outcome, including: Mode of delivery, Haemorrhage, Sepsis and Glycemic control.

c. The effect of low-dose aspirin initiated in the first trimester of diabetes pregnancy on microalbuminuria was evaluated.

Methodology:

A phase III multicentre randomized double-blinded placebo-controlled trial of daily low-dose aspirin 150 mg initiated between 11^{+0} and 13^{+6} weeks' gestational age and continued until 36 weeks' gestation was designed. Participants had a background history of pre-pregnancy type 1 or type 2 diabetes of at least six months duration, and a singleton viable intrauterine pregnancy. Women with a background of vascular disease (cardiovascular, diabetic nephropathy or hypertension) were excluded, as were those for whom aspirin was already recommended based on other risk factors such as early-onset preeclampsia in a previous pregnancy or multifetal gestation. The study was conducted at six university-affiliated perinatology centers across Ireland.

Treatment commenced after a satisfactory first trimester assessment, which included sonographic confirmation of fetal cardiac activity, followed by four-weekly clinical review visits including blood draws for renal profile, with quantification of microalbuminuria. Once a gestational age of 36 weeks was reached, trial medication was discontinued and weekly study visits took place until birth.

Participants took the medication once daily at night with water. Trial staff checked returned medication for discrepancies at dispensing, and discussed non-compliance during study visits. Non-compliance, defined as taking less than 80% of prescribed medication, resulted in trial withdrawal.

Owing to the variation in insulin regimens among study participants (basal bolus regimens and insulin pumps), insulin dosing in units per kilogram of body weight was calculated at each visit in order to facilitate comparison. Glycosylated haemoglobin (HbA1c) was measured at screening, once per trimester and at any other time point in accordance with clinical need.

The planned study sample size used a composite outcome of placental dysfunction consisting of pre-eclampsia, preterm delivery less than 34 weeks' gestation, low birthweight below the 10^{th} centile and perinatal mortality. A total sample size of 566 was required to achieve a 35% reduction in the composite outcome, assuming a 5% (two-sided) type I error

and 80% statistical power (3). However, due to a lower than anticipated recruitment rate during the Covid-19 pandemic, the anticipated trial recruitment was expected to be 360 patients and, finally, only 134 patients were recruited to the trial in total. The primary outcome was therefore underpowered, with a retrospective power calculation of 64%.

The database was anonymized, encrypted and stored in accordance with Data Protection law. Treatment groups were analysed by Intention-to-treat (ITT) analysis - all patients randomized having a composite outcome measure. Data were centrally managed using ClinInfo® and analysed using SAS 9.4®. The two treatment groups were compared using t-tests or chi-square tests, as required, and longitudinal data were compared using a Repeated Measures analysis. A p-value <0.05 was considered statistically significant.

The trial protocol (3) was reviewed and approved by the Health Products Regulatory Authority (HPRA) and National Ethics Committee, (EudraCT number 2018-000770-29) and the trial was registered prospectively on clinicaltrials.gov (trial ID: NCT03574909).

Number of patients (planned and analysed):

Original planned sample size: 600
Analyzed: 134

Diagnosis and main criteria for inclusion:

Diagnosis: Women with type I or type II diabetes of at least 6 months' duration, with a singleton pregnancy in the first trimester.

Inclusion criteria:

- Ability to comprehend the Patient Information Leaflet and to provide signed and dated informed consent.
- Willingness to comply with all study procedures and be available for the duration of the study.
- Female, age >18 years.
- Singleton pregnancy, ongoing at 11 – 13+6 weeks' gestation.
- Pre-pregnancy diagnosis of type I or type II diabetes of at least 6 months' duration.
- Fulfilment of each criterion must be clearly evidenced (in lab reports or correspondence) and/or documented in the medical records

Test product, dose and mode of administration, batch number:

Test product: Tromalyt® 150mg prolong release capsule for oral ingestion. The capsule contained 150mg of anti-platelet agent acetylsalicylic acid, maize starch and Sucrose 20:80. The capsule also contained Copovidone (Kollidon VA-64), Eudragit L, Ethylcellulose and Triacetin. The capsule was made with gelatin, erythrosine, quinoline yellow, and titanium dioxide. Tromalyt® is trademark of Meda Pharma SL (Reg 59.210).

Dose: 150 mg.

Mode of administration: oral.

Batch numbers:

- IP001 – IP468 (supplied by Lab. Sanitatis #VA2.741#)

- IP470 – IP585 (supplied by Lab. Sanitatis #VA3.227#)
- IP586 – IP 705 (supplied by Lab. Sanitatis #VA3.820#)

Duration of treatment: ~ 6 months

Reference therapy*, dose and mode of administration, batch number:

*Please note that within the framework of this clinical trial, a placebo was incorporated as a control to facilitate the comparative analysis of the effects exhibited by the Investigational Medicinal Product. This approach was taken in contrast to utilizing a reference therapy product for the comparative assessment.

Placebo size 0 hard gelatin capsules for oral ingestion, containing 100% of microcrystalline cellulose (Sanitatis®).

Dose: 150 mg.

Mode of administration: oral.

Batch numbers:

- IP001 – IP468 (supplied by Lab. Sanitatis #VA2.741#)
- IP470 – IP585 (supplied by Lab. Sanitatis #VA3.227#)
- IP586 – IP 705 (supplied by Lab. Sanitatis #VA3.820#)

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Criteria for evaluations:

Efficacy:
The primary outcome was a composite clinical measure of placental dysfunction that included preeclampsia (new-onset hypertension after 20 weeks' gestation and new-onset proteinuria or maternal organ dysfunction (1), preterm birth less than 34 weeks, birth weight below the 10th centile (2), or perinatal mortality.

Secondary outcome measures included mode of delivery, neonatal unit admission, and determination of glycemic control including insulin requirements and glycosylated haemoglobin across gestation.

Safety:
Please see Safety Results section below.

Statistical Methods:

Four populations were described and utilized in the statistical analyses:
a. All Randomized
b. Intention-to-treat (ITT): All randomized having a composite outcome measure.
c. Safety Population: All randomized who received at least one dose of study medication.
d. Per-protocol (PP): ITT population excluding major protocol violations and non-compliance with study treatment Clinical outcomes (primary and secondary) were described in the ITT and PP populations.

The primary study outcome variable of placental dysfunction was analyzed using logistic regression with treatment group as a factor. The 5% level of significance and 95% confidence interval was used in the statistical inference, however these results were considered under-powered. Repeated measures analysis were used to analyze data collected at repeat visits. All collected data were summarized with descriptive statistics.

Summary - Conclusions

Efficacy Results:

1. **Primary Outcome:** The composite measure of placental dysfunction did not differ significantly between the aspirin and placebo groups (25% vs. 21%, p = 0.796).
2. **Secondary Outcome:** Aspirin-treated pregnancies exhibited a significant reduction in insulin requirements compared to the placebo group. Insulin dosing in the aspirin group increased by 83% from baseline to 37 weeks of gestation, while the placebo group showed a more substantial increase of 181% in the same period (p = 0.002). Serial HbA1c levels were lower in the aspirin group, although statistical significance was not reached (p = 0.222).

Safety Results:

There were 72 serious adverse events reported in the trial overall, two of which were determined to be serious adverse reactions (Table 1) and two of which were determined to be suspected unexpected serious adverse reactions (SUSARs) (Table 2). The SUSARs were reported to the Eudravigilance Clinical Trial Module by RCSI within the required timeframe.

Table 1

Study ID	SAE Event	Onset Date	IMP related	Outcome Date	Outcome	Serious Criteria	Expected (if related)
6007	Placental haematoma 10x3x1cm resulting in PV bleed & hospitalization	26/05/21	Possibly related	05/10/21	Resolved with Sequelae	IME & Hospitalization	Unexpected
2027	Epigastric left upper quadrant (LUQ) abdominal pain, nausea and vomiting	24/03/22	Possibly related	28/04/22	Resolved without sequelae	Hospitalization	Unexpected

Serious Adverse Reactions case description:

Subject 6007, initially randomized to the placebo arm, had the investigational medicinal product (IMP) permanently stopped after SAE#2. An ultrasound scan on May 26th, 2021, revealed a placental hematoma of 10x3x1 cm. The patient returned on May 27th, 2021, with PV bleeding and was admitted, subsequently being discharged on May 29th, 2021. Diagnostic tests, including two Full Blood Count Tests on May 27th, 2021, showed a decrease in hemoglobin levels from 12 to 11.5. Additionally, a Kleiheur test conducted on the same date showed a volume of fetal cells of less than 2ml, which was not clinically significant. The patient delivered a healthy male infant on October 5th, 2021, via elective caesarean section.

Subject 2027, randomized to the Placebo arm, initially had the IMP interrupted following SAE#2. However, due to patient health improvement, the IMP was permanently stopped. The patient was admitted from home with epigastric left upper quadrant (LUQ) pain and back

discomfort. Pain subsided with Hyoscine, and observations and CTG were reassuring. The patient was discharged on March 26th, with normal OBS and fetal movements. Re-admissions occurred on April 16th and April 25th due to abdominal pain, ongoing nausea, and vomiting. Prescriptions of antiemetic (Prochlorperazine 5mg) and proton pump inhibitor (Omeprazole 40mg) alleviated symptoms, allowing the patient to resume an oral diet. Vital signs remained stable, and the patient was reviewed by the Endocrine team. Discharged on April 28th, the patient was later seen in the Diabetic Clinic on April 29th, where it was decided to interrupt the study IMP to observe if nausea improved by the next clinic review. Lab and diagnostic tests showed normal blood glucose levels and ketones, as well as normal fetal CTG results on March 25th, 2022. Further tests conducted on March 26th, 2022, including full blood count (FBC), urea and electrolytes (U+Es), and liver function tests (LFTs), were within normal ranges.

Table 2

Study ID	SAE Event	Onset Date	IMP related	Outcome Date	Outcome	Serious Criteria	Expected (if related)
1015	Admitted to Mater eye emergency department with retinal haemorrhage. Follow up recurred retinal hemorrhage	04/08/21	Possibly related	02/11/21	Resolved with Sequelae	Hospitalization	Unexpected
2028	DKA, Abruption, Infant IUD	19/05/22	Possibly related	28/11/22	Resolved without sequelae	Hospitalization	Unexpected

Suspected Unexpected Serious Adverse Reaction case description:

Subject 1015, randomized to the Active Treatment arm, had the IMP permanently stopped after SAE#1. The patient experienced retinal haemorrhage on two occasions and was admitted to the Mater Eye Emergency Department. Follow-up revealed recurring retinal haemorrhage, with outcomes resolved but sequelae of blurred vision due to the haemorrhage. Admitted again to Mater Eye Emergency Department, with no current treatment required, the patient was reviewed within two weeks. Subsequent follow-up showed recurrence of retinal haemorrhage in the right eye on October 14th, 2021. The patient underwent laser therapy at the Mater Hospital on October 26th, 2021, with resolution of the issue confirmed on November 2nd, 2021. The Principal Investigator at the Rotunda Hospital site deemed this episode unrelated to the investigational medicinal product.

Subject 2028, enrolled in the Active Treatment arm, had the IMP regimen discontinued due to irregular attendance at Antenatal Clinic appointments. Subsequently, she was admitted to Beaumont Hospital presenting with Diabetic Ketoacidosis (DKA), culminating in an abruption leading to intrauterine fetal demise (IUD). In the maternal interest, a caesarean section was performed.
Prior to admission, the patient was prescribed Fluoxetine 20mg; Antiemetics - Cyclizine 50mg TDS, Prochlorperazine 5mg TDS; Proton Pump Inhibitor (PPI) - 20mg BD; and Antithrombotic: Tinzaparin injection 4500IU every 24 hours.
The final outcome, as noted in the Postnatal Follow-up discharge letter dated November 28th, 2022, depicted a complex clinical course. Upon admission to the Intensive Care Unit (ICU) with diabetic ketoacidosis, the absence of fetal cardiac activity was observed, necessitating a diagnosis of intrauterine fetal demise. Furthermore, clinical examination revealed evidence of placental abruption and associated bleeding. During the caesarean section, a structurally normal

baby girl was delivered.
Subsequently, the patient encountered postnatal complications, notably a diagnosis of pulmonary embolism, leading to initiation of treatment with a Novel Oral Anticoagulant (NOAC). Additionally, she developed a wound hematoma and infection, requiring intervention and monitoring by medical staff. Placental histology findings were not provided. However, the coroner's report attributed the cause of fetal demise to maternal vascular malperfusion, characterized by hypoxia and stress effects. Noteworthy findings included multiple recent or acute parabasal parenchymal haemorrhages and infarcts.
Postnatally, treatment for pulmonary embolism was initiated, and the patient was managed with a NOAC.

Conclusion

In this study, a total of 437 women were screened for recruitment across six centers from February 2020 to August 2022, with 191 meeting eligibility criteria and 134 ultimately being recruited. The COVID-19 pandemic had a limiting impact on achieving the target population. Moreover, it is important to mention that due to funding limitations, enrollment of subjects across all sites concluded in August 2022.

Baseline characteristics were similar between treatment groups. The composite primary outcome occurred in 25% of the aspirin group and 21% of the placebo group, with no statistically significant differences observed for this outcome or its individual components. The overall caesarean birth rate was 70%, with no significant differences in secondary obstetric outcomes between study groups.

Glycaemic control analysis revealed that insulin dosing in the aspirin group increased by 83% at 37 weeks, while the placebo group showed a 181% increase. This difference in glycemic control between aspirin and control groups reached statistical significance. The aspirin group demonstrated a more marked decrease in HbA1c levels compared to the placebo group, although statistical significance was not reached. Women with type 1 diabetes had a more significant decrease in HbA1c than those with type 2.

The data on Total Serious Adverse Events (SAE) in the study reveals comparable percentages between the two groups. The average number of serious adverse events per patient is consistent at 1.4 for both groups, demonstrating a similar safety profile ($p=0.95$). Regarding the causative factors for SAE, there is no significant difference in the occurrence of SAE due to concomitant medication. However, underlying conditions appear to be a predominant cause of SAE in both groups. This suggests that the study interventions have a generally well-tolerated safety profile, with a low incidence of ongoing serious adverse events.
In summary, the study demonstrates similar rates of Total SAEs and average SAEs per patient between the two groups, with underlying conditions being a prominent cause. The resolution outcomes also align, emphasizing the overall safety of the interventions with a low incidence of ongoing SAEs. Improved glycemic control was also associated with a lower risk of adverse perinatal outcome.

In the context of known challenges in perinatal outcomes for pregnancies in women with pregestational diabetes mellitus (PGDM), low-dose aspirin has been explored for preventing complications like preeclampsia and placental dysfunction (4). The study suggests that aspirin may contribute to improved glycaemic control during pregnancy, with lower insulin requirements. The observed reduction in insulin doses may imply a potential beneficial effect on perinatal outcomes.

The study acknowledges limitations, such as the COVID-19 impact on recruitment and the

underpowered primary outcome. Despite the observed improvements in glycaemic control, no significant reduction in macrosomia was noted. The study emphasizes the need for further research to understand the mechanisms underlying the observed effects, with a focus on alternate factors contributing to adverse perinatal outcomes in PGDM.

In conclusion, this double-blinded, placebo-controlled trial, while not achieving statistical significance in the primary outcome, sheds light on the promising impact of aspirin on managing pregnancies complicated by pre-pregnancy diabetes mellitus (PGDM). Beyond its expected antiplatelet properties, aspirin demonstrated a significant reduction in insulin requirements throughout pregnancy compared to the placebo, indicating potential benefits in optimizing glycemic control for women with PGDM.

The findings emphasize the potential of aspirin to play a positive role in PGDM pregnancies, potentially mediated by its influence on glycemic control and beyond its conventional antithrombotic effects.

The study encourages a reconsideration of the initiation of aspirin therapy at the earliest opportunity in PGDM pregnancies, even before conception. Future research, particularly focusing on the pre-conception initiation of aspirin therapy extended until term, holds promise for uncovering its potential to reduce the risk of hyperglycaemia-related teratogenesis and improve pregnancy outcome.

Date of report: 21/02/2024

References:

(1) NICE. Hypertension in pregnancy: diagnosis and management. NICE, NICE; 2019.
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(3) Finnegan C, Dicker P, Fernandez E, Tully E, Higgins M, Daly S, et al. Investigating the role of early low-dose aspirin in diabetes: A phase III multicentre double-blinded placebo-controlled randomised trial of aspirin therapy initiated in the first trimester of diabetes pregnancy. Contemp Clin Trials Commun. 2019;16:100465.
(4) Choi YJ, Shin S. Aspirin Prophylaxis During Pregnancy: A Systematic Review and Meta-Analysis. Am J Prev Med. 2021;61(1):e31-e45.