Aspirin versus placebo in twin pregnancies for preeclampsia prevention: A multicentre, randomised, double-blind, placebo-controlled trial (ASPRE-T)

Version	1.0
Date	16 September 2021
Sponsor	Fundación para la Formación e Investigación Sanitaria
Trial #	FFIS/2019/01/AS
Trial registration	EudraCT Number: 2019-003341-15

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1. ADMINISTRATIVE INFORMATION

This document describes the ASPRE-T trial, sponsored by Fundación para la Formación e Investigación Sanitaria (FFIS). The sponsor will coordinate sites in Spain. The Fetal Medicine Foundation will coordinate sites in Austria, Belgium, Bulgaria, Denmark, Germany, Greece, Hungary, Ireland, Italy, Poland and Portugal and the UK. Sites in Argentina, Australia, Brazil and Israel will use a parallel protocol but will have their own sponsor and be coordinated locally. Data from all sites will be combined for the final trial publication.

This protocol provides information about procedures for entering participants into the trial, the background, rationale, objectives, trial population, intervention, methods, statistical analyses, ethical considerations, dissemination plans and administration of the trial. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to registered investigators in the trial. Sites entering participants for the first time should confirm they have the correct version through a member of the trial team at their coordinating organisation.

The FFIS and FMF support the commitment that its trials adhere to the SPIRIT guidelines. As such, the protocol template is based on an adaptation of the Medical Research Council protocol template (2012) and the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2012 Statement for protocols of clinical trials (Chan et al, 2013a). The SPIRIT Statement Explanation and Elaboration document (Chan et al, 2013b) can be referred to for further detail about specific items.

1.1 Compliance

The trial will be conducted in compliance with the approved protocol, the Declaration of Helsinki (2008), the principles of Good Clinical Practice (GCP) as laid down by the Commission Directive 2005/28/EC with implementation in national legislation in the UK by Statutory Instrument 2004/1031 and subsequent amendments, the Human Tissue (Quality and Safety for Human Application) Regulations 2007, the UK Data Protection Act 2018, the EU General Data Protection Regulation (GDPR) 2016, and the National Health Service (NHS) UK Policy Framework for Health and Social Care. International sites will comply with the principles of GCP as laid down by ICH topic E6 (Note for Guidance on GCP), Commission Directive 2005/28/EC, the European Directive 2001/20/EC (where applicable), the EU Tissue and Cells Directives 2004/23/EC, 2006/17/EC and 2006/86/EC, and other national and local applicable regulations.

Agreements that include detailed roles and responsibilities will be in place between participating sites and the Sponsor.

Participating sites in the UK, Ireland, Germany, Belgium, Denmark, Bulgaria, Greece, Hungary, Portugal, Poland, Italy, Austria, Israel, Argentina, Brazil and Australia will inform the FMF and sites in Spain will inform the FFIS as soon as they are aware of a possible serious breach of GCP, so that the FMF or FFIS can fulfil its requirement to report the breach if necessary within the timelines specified in the UK Clinical Trials Regulations (currently 7 days). For the purposes of this regulation a 'serious breach' is a breach of the conditions and principles of GCP in connection to the trial or the trial protocol that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the participants in the trial, or
- The scientific value of the trial.

1.2 Sponsor

FFIS is the trial sponsor and will coordinate the participating sites in Spain. The sponsor has delegated responsibility for coordination of ASPRE-T trial to the FMF for sites in Austria, Belgium, Bulgaria, Denmark, Germany, Greece, Hungary, Ireland, Italy, Poland and Portugal and the UK. Queries relating to FFIS sponsorship of this trial should be addressed via the relevant local coordinating organisation.

1.3 Structured trial summary

Primary Registry and Trial Identifying Number	EudraCT Number: 2019-003341-15
Source of Monetary or Material Support	Fetal Medicine Foundation
Sponsor	Fundación para la Formación e Investigación Sanitaria (FFIS)
Coordinating Organisations	Fundación para la Formación e Investigación Sanitaria – Spain The Fetal Medicine Centre: Austria, Belgium, Bulgaria, Denmark, Germany, Greece, Hungary, Ireland, Italy, Poland, Portugal and the UK.
Contact for Scientific Queries	Dr Catalina De Paco Fetal Medicine Unit, Hospital Universitario "Virgen de la Arrixaca", Murcia, Spain. Telephone: +34 676672617 Email: katy.depaco@gmail.com Prof Kypros Nicolaides Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, SE5 8BB, UK. Telephone: +44 2032998256 Email: kypros@fetalmedicine.com
Public Title	Aspirin in the prevention of preeclampsia in twin pregnancies
Scientific Title	Aspirin versus placebo in the prevention of preterm preeclampsia in twin pregnancies
Countries of Recruitment	Sponsored by Fundación para la Formación e Investigación Sanitaria: Austria, Belgium, Bulgaria, Denmark, Germany, Greece, Hungary, Ireland, Italy, Poland, Portugal, Spain and the UK. Local sponsorship, running a parallel protocol: Argentina, Australia, Brazil and Israel
Health Condition(s) or Problem(s) Studied	Preeclampsia
Screening	All women with dichorionic diamniotic (DCDA) and monochorionic diamniotic (MCDA) twin pregnancies attending the first trimester scan at 11^{+2}-13^{+6} weeks' gestation.
Intervention(s)	Participants will be randomised into one of two arms of the trial. Randomised participants will be advised to start 150 mg aspirin or placebo tablets once per day until 36^{+0} weeks' gestation or when signs of labour commence. The maximum duration for aspirin or placebo intake will be 174 days.
Sample size	2400
Participant inclusion criteria	• Age > 18 years; • DCDA or MCDA twin pregnancies; • Both live fetuses at 11^{+2}-13^{+6} weeks of gestation; • Informed and written consent.
Participant exclusion criteria	• Monoamniotic twins • Triplet pregnancies that had undergone embryo reduction to twins or with one vanishing twin

	• Pregnancies complicated by major fetal abnormality or nuchal translucency thickness >3.5 mm identified at the 11^{+2}-13^{+6} weeks scan; • MCDA twin pregnancies in which there are early signs of TTTS or sFGR defined by a 20% discordance in CRL at the 11^{+2}-13^{+6} weeks' scan; • Women who are unconscious or severely ill, those with learning difficulties, or serious mental illness; • Women taking low-dose aspirin regularly (administration must have ceased >7 days prior to randomization); • Participation in another drug trial within the previous 7 days; • Haemorrhagic diathesis; coagulation disorders such as haemophilia and thrombocytopenia or concurrent anticoagulant therapy; • Active or history of recurrent peptic ulceration and/or gastric/intestinal haemorrhage, or other kinds of bleeding such as cerebrovascular haemorrhages; • Patients who are suffering from known gout, severe hepatic impairment or severe renal impairment; • Hypersensitivity to salicylic acid compounds or prostaglandin synthetase inhibitors (e.g. certain asthma patients who may suffer an attack or faint and certain patients who may suffer from bronchospasm, rhinitis and urticaria) or to any excipients (see section 6.1 of the SmPC for details); • Patients on long term non-steroidal anti-inflammatory medication; • Not fluent in local language and absence of interpreter • Any other reason the clinical investigators think will prevent the potential participant from complying with the trial protocol.
Primary Outcome(s)	To determine the effect of low-dose aspirin on the incidence of preterm preeclampsia with delivery <37 weeks' gestation.
Key Secondary Outcomes	To determine the effect of low-dose aspirin on the incidence of (stratified according to chorionicity) : ○ Delivery with PE at <32 weeks, <34 weeks, <37 weeks and at any gestation. ○ Features of severe PE including: ○ stroke ○ Eclamspia ○ systolic blood pressure >160 mmHg on at least one occasion ○ diastolic blood pressure >110 mmHg on at least one occasion ○ respiratory failure requiring intubation or mechanical ventilation ○ myocardial ischemia or infarction ○ pulmonary edema ○ hepatic dysfunction (INR >1.2 in the absence of DIC) ○ hepatic hematoma or rapture ○ platelet count <100 x 109/litre ○ abnormal liver function enzymes (ALT or AST >67 iu/litre) ○ acute kidney injury ○ creatinine >150 µmol/L ○ cortical blindness

	○ retinal detachment ○ transfusion of any blood products ○ HELLP syndrome ○ placental abruption ○ postpartum hemorrhage (defined as blood loss ≥1 L within the first 24 hours after birth) ○ intensive therapy or high-dependency unit admission ○ intubation and mechanical ventilation ○ confirmed sepsis (positive blood or urine cultures) up to post-natal discharge ○ total number of nights in hospital ○ Gestational hypertension (GH) ○ Birth at <32 weeks, <34 weeks and <37 weeks ○ Spontaneous ○ Iatrogenic for PE, GH or FGR ○ Iatrogenic for other reason ○ Death of one twin and / or both twins before discharge from hospital ○ Miscarriage of the whole pregnancy or death of one twin <24 weeks' gestation. ○ Stillbirth or neonatal death of one or both twins at <32 weeks, <34 weeks, <37 weeks and at any gestation. ○ Birthweight <3rd, <5th and <10th percentile for gestational age. ○ Placental abruption (clinically or on placental examination) at <32 weeks, <34 weeks, <37 weeks and at any gestation. ○ Postpartum hemorrhage (defined as blood loss ≥1 L within the first 24 hours after birth). ○ Neonatal morbidity ○ Intraventricular hemorrhage (IVH) grade II or above – Defined as bleeding into the ventricles ▪ Grade II (moderate) – IVH occupies <50% of the lateral ventricle volume ▪ Grade III (severe) – IVH occupies ≥50% of the lateral ventricle volume ▪ Grade IV (severe) – Hemorrhagic infarction in periventricular white matter ipsilateral to a large IVH ○ Neonatal sepsis confirmed bacteremia in cultures ○ Encephalopathy grade (mild, moderate, severe) ○ Neonatal seizures ○ Anemia defined as low hemoglobin and / or hematocrit requiring blood transfusion ○ Respiratory distress syndrome defined as need of ventilation with or without surfactant ○ Necrotizing enterocolitis requiring surgical intervention ○ Composite of any of the above ○ Neonatal therapy ○ Neonatal intensive care unit admission ○ Ventilation defined as need of positive pressure (continuous positive airway pressure (CPAP) or nasal continuous positive airway pressure (NCPAP)) or intubation ○ Composite of any of the above ○ Length of stay in neonatal intensive care unit

1.4 Roles and responsibilities

Protocol contributors

Name	Affiliation	Role
Catalina De Paco	Fetal Medicine Unit, Hospital Universitario "Virgen de la Arrixaca", Murcia, Spain.	Chief Investigator
Kypros Nicolaides	Fetal Medicine Research Institute, King's College Hospital, London, UK	Co-Chief Investigator
David Wright	Institute of Health Research, University of Exeter, Exeter, UK.	Trial statistician
Argyro Syngelaki	Fetal Medicine Research Institute, King's College Hospital, London, UK	Trial Research Coordinator
Jonathan Lai	Fetal Medicine Research Institute, King's College Hospital, London, UK	Trial Research Coordinator
Ann Tabor	The Juliane Marie Centre, Rigshospitalet, Copenhagen University Hospital and the University of Copenhagen, Denmark.	Site Principal Investigator
Hamutal Meiri	Bar Ilan University, Ramat Gan, Tel Aviv, Israel	Trial Research Associate
Kate Maclagan	Fetal Medicine Foundation	Clinical Project Manager
Ana Sanchez	Fetal Medicine Foundation	Trial Manager

Role of trial sponsor

Name	Affiliation	Role
Maria Fuensanta Martinez Lozano	Fundación para la Formación e Investigación Sanitaria	Regulatory sponsor

Trial Management Group

Name	Affiliation	Role and responsibilities
Catalina De Paco	Fetal Medicine Unit, Hospital Universitario "Virgen de la Arrixaca", Murcia, Spain.	Chief Investigator. Responsible for the concept and design of the study protocol, application for ethics and drug agency approval, supervision of the research teams, coordination and management of the study in Spain.
Kypros Nicolaides	Fetal Medicine Research Institute, King's College Hospital, London, UK	Co-Chief investigator. Responsible for the concept and design of the study protocol, application for ethics and drug agency approval, supervision of the research teams, coordination and management of the study in sites other than Spain. Writing up the scientific publications.
David Wright	Institute of Health Research, University of Exeter, Exeter, UK.	Statistician. Responsible for statistical analysis and monitoring of data.
Argyro Syngelaki	Fetal Medicine Research Institute, King's College Hospital, London, UK	Trial Research Coordinator. Responsible for coordination of data collection and monitoring of blinded data for all the centres.

Jonathan Lai	Fetal Medicine Research Institute, King's College Hospital, London, UK	Trial Research Coordinator. Supervising the research teams, coordination and management of the study sites.
Kate Maclagan	Fetal Medicine Foundation, UK	Clinical Project Manager.
Ana Sanchez	Fetal Medicine Foundation, UK	Trial Manager

Trial Steering Committee

Name	Affiliation	Role and responsibilities
Marietta Charakida	Pediatric cardiology, St Thomas's University Hospital, London, UK.	Chair. Independent of the trial team
Jacques Jani	Department of Obstetrics & Gynecology, CHU Brugmann, Belgium, Brussels	Independent Specialist in Obstetrics and Fetal Medicine
Catalina De Paco	Fetal Medicine Unit, Hospital Universitario "Virgen de la Arrixaca", Murcia, Spain.	Chief Investigator
Kypros Nicolaides	Fetal Medicine Research Institute, King's College Hospital, London, UK	Co-Chief Investigator

Independent Data Monitoring Committee

Name	Affiliation	Role and responsibilities
Christina Yu	Fetal Medicine Unit, Imperial College Healthcare NHS Trust, UK.	Chair. Independent of the trial team.
Ian Bradbury	Statistics at Frontier Science Scotland, UK.	Independent Statistician. Responsible for monitoring and statistical analysis of data.
Alexandros Sotiriadis	Department of Obstetrics & Gynecology, Aristotle University of Thessaloniki, Greece.	Independent Specialist in Obstetrics and Fetal Medicine
Anca Panaitescu	Fetal Medicine Department Filantropia Hospital Bucharest, Romania.	Independent Specialist in Obstetrics and Fetal Medicine

Other Partners

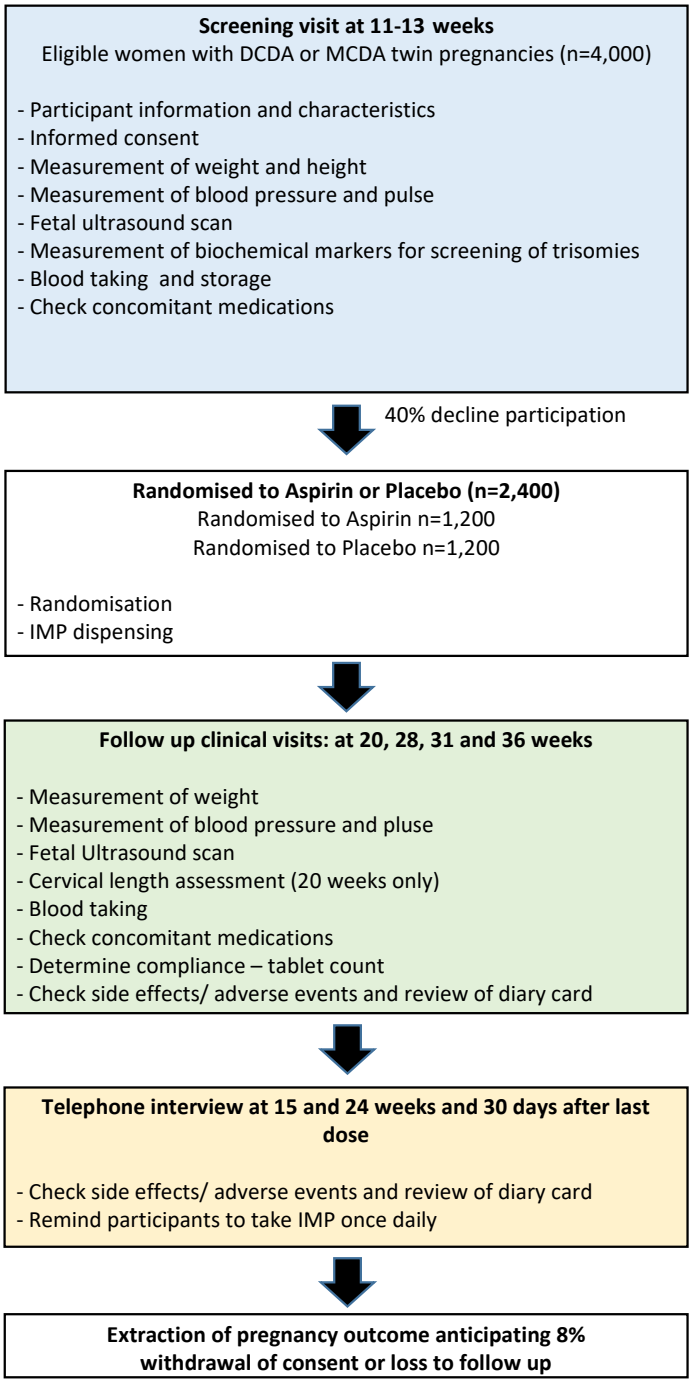
Name	Role and responsibilities
GSTT Pharmaceuticals	GSTT Pharmaceuticals will be responsible for providing the language verification service for labels only and will manage the outsourcing of the IMP manufacture. RenaClinical is responsible for manufacture, packaging, labeling and distribution of the IMP. Contact person GSTT: Name: Kate Hadavizadeh, Postal Address: Guy's & St Thomas' NHS Foundation Trust Pharmacy Manufacturing Unit, 13th Floor Tower Wing, Guy's Hospital, Great Maze Pond, London, SE1 9RT

	Telephone No.: 0207 188 7188 Ext: 51674, Email: Kate.Hadavizadeh@gstt.nhs.uk and PMUclinicaltrials@gstt.nhs.uk Contact person RenaClinical: Name: Peter Mollison, Postal Address: Unit 11 Gatwick Metro Centre, Horley, RH6 9GA Telephone No.: 01293368080 Email: peter@renaclinical.com
Sealed Envelope	Sealed Envelope will be responsible for preparation of the IMP code lists and the provision of patient randomisation online and code breaking by authorised users online and via telephone.

2. VISIT SCHEDULE AND TRIAL DIAGRAM

	Routine Screening Visit	Randomisation Visit	Follow up clinical visits	Telephone interview
Gestation (weeks)	11^{+2}-13^{+6}or CRL 45-84mm	11^{+2}-14^{+3}	20^{+0}-21^{+6}, 28^{+0}-29^{+6}, 31^{+0}-32^{+6}, 35^{+0}-36^{+6}	15^{+0}-16^{+6}, 24^{+0}-25^{+6} 30 days after last dose of IMP
Participant information and characteristics	√			
Informed consent	√			
Measurement of weight and height*	√		√	
Measurement of blood pressure and pulse	√		√	
Fetal ultrasound scan	√		√	
Uterine artery pulsatility index	√		√	
Measurement of biochemical markers for screening of trisomies	√			
Blood taking and storage for research (1 x serum, 1 x EDTA, 1 x citrate)**	√		√ at 20^{+0}-$21^{+6}$and 31^{+0}-32^{+6} weeks	
Check concomitant medications***	√		√	√
Randomisation		√		
IMP dispensing		√ - 2 bottles		
Ensure compliance – tablet count at clinical visits only			√	√
Check side effects/ adverse events and review of diary card			√	√
Discontinue and retrieve IMP if visit scheduled at 36+4 – 36+6			****Last visit	

* height at first visit only;
** in centers with such capacity
***including COVID-19 vaccination status
**** If visit scheduled 35+0 – 36+3 perform tablet count but do not retrieve IMP – ask participant to dispose of any remaining IMP following their dose at 36+6


Screening visit at 11-13 weeks
Eligible women with DCDA or MCDA twin pregnancies (n=4,000)
- Participant information and characteristics
- Informed consent
- Measurement of weight and height
- Measurement of blood pressure and pulse
- Fetal ultrasound scan
- Measurement of biochemical markers for screening of trisomies
- Blood taking and storage
- Check concomitant medications
40% decline participation
Randomised to Aspirin or Placebo (n=2,400)
Randomised to Aspirin n=1,200
Randomised to Placebo n=1,200
- Randomisation
- IMP dispensing
Follow up clinical visits: at 20, 28, 31 and 36 weeks
- Measurement of weight
- Measurement of blood pressure and pluse
- Fetal Ultrasound scan
- Cervical length assessment (20 weeks only)
- Blood taking
- Check concomitant medications
- Determine compliance – tablet count
- Check side effects/ adverse events and review of diary card
Telephone interview at 15 and 24 weeks and 30 days after last dose
- Check side effects/ adverse events and review of diary card
- Remind participants to take IMP once daily
Extraction of pregnancy outcome anticipating 8% withdrawal of consent or loss to follow up

3. ABBREVIATIONS

AE	Adverse Event	PE	Preeclampsia
AR	Adverse Reaction	PI	Principal Investigator
ß-hCG	ß-human chorionic gonadotropin	PIS	Participant Information Sheet
CI	Chief Investigator	QA	Quality Assurance
CRF	Case Report Form	QC	Quality Control
CRL	Crown-rump length	QP	Qualified person
CTA	Clinical Trial Authorisation	R&D	Research and Development
DMC	Data Monitoring Committee	REC	Research Ethics Committee
DCDA	Dichorionic Diamniotic	RR	Relative risk
DSUR	Development Safety Update Report	SAE	Serious Adverse Event
FDA	US Food and Drug Administration	SAP	Statistical Analysis Plan
FMF	Fetal Medicine Foundation	SAR	Serious Adverse Reaction
FWA	Federal Wide Assurance	SCBU	Special Care Baby Unit
GCP	Good Clinical Practice	SOP	Standard Operating Procedure
GH	Gestational hypertension	SPC	Summary of Product Characteristics
ICH	International Conference on Harmonisation	SSA	Site Specific Approval
IDMC	Independent data monitoring committee	sFGR	Selective fetal growth restriction
IMP	Investigational Medicinal Product	SUSAR	Suspected Unexpected Serious Adverse
ITT	Intention-to-Treat	TMF	Trial Master File
MCDA	Monochorionic Diamniotic	TMG	Trial Management Group
MHRA	Medicines and Healthcare Products Regulatory	ToR	Terms of Reference
NREC	National Research Ethics Committee	TSC	Trial Steering Committee
NICU	Neonatal Intensive Care Unit	TTTS	Twin-to-twin transfusion syndrome
NT	Nuchal translucency	UTA PI	Uterine artery pulsatility index
PAPP-A	Pregnancy associated plasma protein-A		

4. GLOSSARY OF TERMS

- Adverse Outcome - a harmful outcome that is usually indicated by some result such as morbidity, mortality.
- Algorithm - a formula or set of steps for solving a particular problem.
- Crown-Rump length - the measurement of the length of human embryos and fetuses from the top of the head (crown) to the bottom of the buttocks (rump).
- Detection rate - the frequency of discovering a particular outcome from an analysis.
- Dichorionic diamniotic – twins that each have their own sac and placenta.
- False positive rate - the frequency with which a test indicates that a given condition is present when it is not.
- Fetus - the name for an embryo after 8 weeks of development
- Meta-analysis - a statistical method which can be used to combine the results of two or more studies.
- Monochorionic diamniotic- twins that share a single placenta but have separate sacs.
- Neonatal morbidity - a diseased condition or state during the first 28 days of life.
- Neonatal mortality - the death rate during the first 28 days of life.
- Neonatal death – the death of a live born infant (irrespective of how early in gestation it was born) within the first 28 days of life.
- Nuchal translucency - the thickness at the back of the fetal neck, usually measured at the first trimester scan. Measuring this thickness helps assess the risk of Down's syndrome and other abnormalities.
- Participant demographics – objective characteristics of a population; e.g. age, racial origin, present or prior disease.
- Perinatal –the period immediately before, during and after birth.
- Placebo - an inactive substance or preparation used as a control in an experiment or test to determine the effectiveness of a medicinal drug.
- PPROM – Preterm Premature Rupture of the Membranes. A situation in which the amniotic membrane surrounding the baby becomes ruptured before 37 weeks' gestation.
- Preterm delivery – delivery of a fetus at <37 weeks' gestation.
- Prophylactic use – the use of a medication or a treatment designed to prevent a disease from occurring.
- Prospective study – a study in which the subjects are first identified and then followed forward as time passes in order to try and identify factors that influence the development of a particular outcome (disease)
- Randomised control trial - a study in which a number of similar people are randomly assigned to two (or more) groups to test a specific drug or treatment. One group (the experimental group) receives the treatment being tested, the other (the comparison or control group) receives an alternative treatment, a dummy treatment (placebo) or no treatment at all. The groups are followed up to see how effective the experimental treatment was.
- Relative risk - the ratio of the risk of disease or death among those exposed to certain conditions compared with the risk for those who are not exposed to the same conditions. If both groups face the same level of risk, the relative risk is 1. If the first group had a relative risk of 2, participants in that group would be twice as likely to have the event happen. A relative risk of less than one means the outcome is less likely in the first group.
- Retrospective study - a study that looks backwards and examines exposures to suspected risk or protection factors in relation to an outcome that has already happened and is established at the start of the study.
- Screening – a process that aims to identify people at increased risk of a particular disease or outcome.
- Selective fetal growth restriction – a complication of twins with unequal sharing of placenta
- Stillbirth – death of a fetus in utero at ≥24 weeks of gestation.
- Twin-to-twin transfusion syndrome – a complication of twins sharing a placenta with unequal sharing of blood flow between the two twins

INTRODUCTION

5.1 Background

Singleton pregnancies

Preeclampsia (PE) which results in premature birth is a serious complication of pregnancy and a major cause of short and long-term maternal and perinatal morbidity and mortality (Witlin et al., 2000, Irgens et al., 2001, von Dadelszen et al., 2003, Yu et al., 2008). Extensive research in the last decade has resulted in the development of a method for first-trimester prediction of subsequent development of PE in singleton pregnancies from a combination of maternal characteristics and medical history with uterine artery pulsatility index (UtA-PI), mean arterial pressure (MAP) and serum placental growth factor (PLGF); with this method we can predict about 90% of early-PE with delivery at <32 weeks' gestation and 75% of preterm-PE with delivery at <37 weeks, at screen positive rate (SPR) of 10% (Wright et al., 2015, O'Gorman et al., 2016).

A subsequent multicentre trial using this algorithm found that prophylactic use of aspirin (150 mg / day from 11-14 until 36 weeks' gestation) reduces the incidence of early-PE by 89% and preterm-PE by 62% (Rolnik et al., 2017). A systematic review and meta-analysis on the prophylactic use of aspirin involving 18,907 pregnancies reported that administration of aspirin in women at high-risk of developing PE was associated with a significant reduction in the risk of preterm-PE (relative risk 0.62, 95% CI 0.45 to 0.87), but there was no significant effect on term-PE (RR: 0.92, 95% CI 0.70 to 1.21) (Roberge et al., 2018). The reduction in preterm-PE was confined to the subgroup in which aspirin was initiated at ≤16 weeks' gestation and at a daily dose of ≥100 mg (RR: 0.33, 95% CI 0.19 to 0.57) (Roberge et al., 2018).

Twin pregnancies

Twins account for 3-4% of all births and over the last two decades the rate of twin pregnancies has increased steadily essentially due to two reasons: first, widespread use of assisted reproductive techniques and ovulation medications, and second, the increasing age of women when they conceive (Heino et al., 2016). In twin pregnancies, the rate of PE is about 9%, which is 3-times higher than in singleton pregnancies. However, twins are delivered at an earlier gestational age than singletons and consequently comparison of the overall rates of PE between twin and singleton pregnancies underestimates the relative risk of preterm-PE in twins which is 9-times higher (Francisco et al., 2017a, Francisco et al., 2017b, Fox et al., 2014). The same model for first-trimester prediction of PE developed in singleton pregnancies has been adapted for screening in twins; however, screening in a mixed population of singleton and twin pregnancies has demonstrated that use of risk cut-off resulting in SPR of 10% in singletons would result in SPR of 75% in twins with detection rates of about 75% for singletons and 99% for twins (Francisco et al., 2017b). In this respect all twin pregnancies can be considered to be at high-risk of preterm-PE and therefore in any potentially preventative intervention study all twins can be included.

A systematic review on the prophylactic use of aspirin in multiple pregnancies identified five trials. Use of aspirin was not associated with reduction in the incidence of PE in any of the trials but a meta-analysis of the trials, in a combined total of 898 pregnancies, reported that first, aspirin reduced the incidence of mild-PE (RR 0.44, 95% CI 0.24 to 0.82) but not severe-PE (RR 1.02, 95% CI 0.61 to 1.72), and second, there was significant reduction in PE if aspirin was initiated >16 weeks' gestation (RR 0.64, 95% CI 0.43 to 0.96) but not <16 weeks (RR 0.86, 95% CI 0.41 to 1.81) (Bergeron et al., 2016). These results are inconsistent with findings in singleton pregnancies. The authors recommended that additional studies are required before recommending that low-dose aspirin should be initiated early in pregnancy for all multiple gestations.

5.2 Safety of low dose aspirin

The relative safety of first-trimester use of low-dose aspirin has been demonstrated in large cohort and case control studies, which reported that the drug is not associated with increase in risk of congenital heart

defects or other structural or developmental anomalies (Slone et al., 1976; Klebanoff and Berendes, 1988; Werler et al., 1989; Norgard et al., 2005).

Potential risks associated with aspirin therapy during the third-trimester include haemorrhagic complications to the mother or fetus and premature closure of the ductus arteriosus for the fetus.

Randomised studies reported that although approximately 10% of women receiving low-dose aspirin complained of gastro-intestinal symptoms there was no evidence of increase in any type of maternal bleeding (Sibai et al., 1993; Caritis et al., 1998; Rotchell et al., 1998). Similarly, the best evidence suggests that low-dose aspirin started before 16 weeks' gestation does not increase the risk of placental abruption (RR: 0.62, 95% CI: 0.08–5.03; Bujold et al., 2010). No additional adverse effects related to epidural anaesthesia have been reported in women taking low-dose aspirin compared to those taking placebo (Sibai et al., 1995).

Prospective and case-control studies did not find an association between daily consumption of 60-150 mg of aspirin during the third-trimester and antenatal closure of the ductus arteriosus (Di Sessa et al., 1994; Schiessl et al., 2005; Wyatt-Ashmead, 2011). A meta-analysis including more than 26,000 women randomised to low-dose (80-150 mg) aspirin or placebo/no treatment during pregnancy demonstrated that the use of aspirin was not associated with an increase in intra-ventricular haemorrhage or other neonatal bleeding (Duley et al., 2007).

In the trials evaluating the effect of aspirin for the prevention of PE in high-risk women, treatment was continued until a pre-specified gestation between 34 and 37 weeks or until delivery. On the basis of currently available evidence it would be reasonable to continue with low-dose aspirin well into the third-trimester of pregnancy.

5.3 Objectives

To examine if the prophylactic use of low-dose aspirin from the first-trimester of pregnancy in women with twin pregnancy can reduce the incidence of PE with delivery <37 weeks' gestation.

Primary objective:

To determine the effect of low-dose aspirin on the incidence of PE with delivery <37 weeks' gestation.

Secondary objectives:

To determine the effect of low-dose aspirin on the incidence of (stratified according to chorionicity) :

- Delivery with PE at <32 weeks, <34 weeks, <37 weeks and at any gestation.
- Birth at <32 weeks, <34 weeks and <37 weeks
  - Spontaneous
  - Iatrogenic for PE, GH or FGR
  - Iatrogenic for other reason
- Death of one twin and / or both twins before discharge from hospital
  - Miscarriage of the whole pregnancy or death of one twin <24 weeks' gestation.
  - Stillbirth or neonatal death of one or both twins at <32 weeks, <34 weeks, <37 weeks and at any gestation.
- Birthweight <3rd, <5th and <10th percentile for gestational age.
- Placental abruption (clinically or on placental examination) at <32 weeks, <34 weeks, <37 weeks and at any gestation.
- Postpartum hemorrhage (defined as blood loss ≥1 L within the first 24 hours after birth).
- Neonatal morbidity
  - Intraventricular hemorrhage (IVH) grade II or above – Defined as bleeding into the ventricles
    - Grade II (moderate) – IVH occupies <50% of the lateral ventricle volume
    - Grade III (severe) – IVH occupies ≥50% of the lateral ventricle volume
    - Grade IV (severe) – Hemorrhagic infarction in periventricular white matter ipsilateral to a large

IVH
- Neonatal sepsis confirmed bacteremia in cultures
- Anemia defined as low hemoglobin and / or hematocrit requiring blood transfusion
- Respiratory distress syndrome defined as need of surfactant and ventilation
- Necrotizing enterocolitis requiring surgical intervention
- Composite of any of the above

- Neonatal therapy
  - Neonatal intensive care unit admission
  - Ventilation defined as need of positive pressure (continuous positive airway pressure (CPAP) or nasal continuous positive airway pressure (NCPAP)) or intubation
  - Composite of any of the above
- Length of stay in neonatal intensive care unit

5.4 Trial design

Proposed study

This is a double-blind randomised placebo-controlled trial. The trial will be conducted in compliance with the protocol, the Declaration of Helsinki (1996), the principles of Good Clinical Practice (GCP) and applicable regulatory requirements. The study will be reviewed and approved by the Research Ethics Committees (REC) and competent authorities in all member states concerned as well as applicable Hospital Management Boards. The FMF and FFIS will manage the sponsors' responsibilities and Quality Assurance to ensure compliance with the Clinical Trial Regulations for sites sponsored by FFIS.

This is a multicentre trial. In the participating centres, all eligible women with DCDA or MCDA twin pregnancy attending for their routine first hospital visit in pregnancy at 11-13 weeks' gestation will be invited to participate in the trial. In this visit we will record maternal characteristics and medical history and perform an ultrasound scan to determine chorionicity (Sepulveda et al., 1996), confirm gestational age from the measurement of the fetal crown- rump length (CRL) (Robinson and Fleming, 1975) of the bigger twin, diagnose any major fetal abnormalities (Syngelaki et al., 2011), screen for chromosomal abnormalities based on the first-trimester combined test (Snijders et el., 1998, Kagan et al., 2008). The Principal Investigators for each site are fetal medicine experts who follow the Fetal Medicine Foundation (FMF) guidelines on how to undertake the appropriate measurements.

6. METHODS

6.1 Site Selection

For sites that have collaborated with the CI and Co-CIs on IMP studies previously, no assessment will be performed. For site(s) that have not collaborated with the CI and Co-CIs on IMP studies, a site assessment will be performed (on-site or remote).

Study Setting

This is a multicentre study that will be carried out in the Fetal Medicine Units in Austria, Belgium, Bulgaria, Denmark, Germany, Greece, Hungary, Ireland, Italy, Poland, Portugal, Spain and the UK. Sites in these countries will be sponsored by FFIS.

Sites in Argentina, Australia, Brazil and Israel will also participate and run a parallel protocol but be sponsored locally.

Site / investigator eligibility criteria

Once a site has been assessed as being suitable to participate in the trial, the trial team will provide them with a copy of this protocol and relevant reference safety information.

To participate in the ASPRE-T trial, investigators and trial sites must fulfil a set of criteria that have been agreed by the TMG. The TMG will issue the Trial Master File (TMF) documentation to use when applying for Site-Specific Approval (SSA).

Principal Investigator's (PI) Qualifications and Agreements

The investigator(s) must be willing to sign a Clinical Trial Site Agreement and an Investigator Agreement to comply with the trial protocol (confirming their specific roles and responsibilities relating to the trial, and that their site is willing and able to comply with the requirements of the trial). This includes confirmation of appropriate qualifications, familiarity with the appropriate use of any investigational products, agreement to comply with the principles of GCP, to permit monitoring and audit as necessary at the site, and to maintain documented evidence of all staff at the site who have been delegated significant trial related duties. The PI may delegate the ability to consent participants to non-medical staff (research midwives, midwifery support workers) if they feel that they have the required competencies (training, education and experience) to do so.

Resourcing at site

The investigator(s) should be able to demonstrate a potential for recruiting the required number of suitable participants within the agreed recruitment period (i.e. the investigators regularly treat the target population). They should also have an adequate number of qualified staff and facilities available for the foreseen duration of the trial to enable them to conduct the trial properly and safely.

Sites will be expected to complete a delegation of responsibilities log and provide staff contact details. The site should have sufficient data management resources to allow prompt data return to the data management team.

6.2 Site approval and activation

The Clinical Trial Authorisation (CTA) for the trial requires that competent authorities are supplied with the names and addresses of all participating site Principal Investigators in their member state. Trial staff will perform this task.

On receipt of the signed Clinical Trial Site Agreement and Investigator Agreement, approved delegation of responsibilities log and staffs contact details, written confirmation will be sent to the site PI. The trial manager or delegate will notify the PI in writing of the plans for site initiation.

The site must conduct the trial in compliance with the protocol as agreed by the Sponsor and, by the regulatory authority(ies) (as appropriate), and which was given a favourable opinion by the Research Ethics Committee (REC). The PI or delegate must document and explain any deviation from the approved protocol, and communicate this to their coordinating organisation. This is with the exception of visits conducted out-of-window or missed visits which do not need to be reported as a deviation. Data on out-of-window and missed visits can be ascertained from the eCRF and will be monitored centrally.

6.3 Participants

Participant selection

There will be **NO EXCEPTIONS** (waivers) to eligibility requirements at the time of randomisation. Questions about eligibility criteria should be addressed PRIOR to attempting to randomise the participant.

The eligibility criteria for this trial have been carefully considered and are the standards used to ensure that only medically appropriate participants are entered. Participants not meeting the criteria should not be entered into the trial for their safety and to ensure that the trial results can be appropriately used to make future treatment decisions for other people with similar diseases or conditions. It is therefore vital that exceptions are not made to these eligibility criteria.

Participants will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

Participant inclusion criteria

- Age $\geq$ 18 years;
- DCDA or MCDA twin pregnancies;
- Both live fetuses at 11^{+2}-13^{+6} weeks of gestation;
- Informed and written consent.

Participant exclusion criteria

- Monoamniotic twins
- Triplet pregnancies that had undergone embryo reduction to twins or with one vanishing twin
- Pregnancies complicated by major fetal abnormality or nuchal translucency thickness >3.5 mm identified at the 11+2-13+6 weeks scan;
- MCDA twin pregnancies in which there are early signs of TTTS or sFGR defined by a 20% discordance in CRL at the 11+2-13+6 weeks' scan;
- Women who are unconscious or severely ill, those with learning difficulties, or serious mental illness;
- Women taking low-dose aspirin regularly (administration must have ceased >7 days prior to randomization);
- Participation in another drug trial within the previous 7 days;
- Haemorrhagic diathesis; coagulation disorders such as haemophilia and thrombocytopenia or concurrent anticoagulant therapy;
- Active or history of recurrent peptic ulceration and/or gastric/intestinal haemorrhage, or other kinds of bleeding such as cerebrovascular haemorrhages;
- Patients who are suffering from known gout, severe hepatic impairment or severe renal impairment;
- Hypersensitivity to salicylic acid compounds or prostaglandin synthetase inhibitors (e.g. certain asthma patients who may suffer an attack or faint and certain patients who may suffer from bronchospasm, rhinitis and urticaria) or to any excipients (see section 6.1 of the SmPC for details);
- Patients on long term non-steroidal anti-inflammatory medication;
- Not fluent in local language and absence of interpreter
- Any other reason the clinical investigators think will prevent the potential participant from complying with the trial protocol.

Eligibility criteria for individuals performing the interventions
All centres involved in the data collection will have staff who are appropriately trained in obstetric ultrasound and possess certificates of competence from the FMF.

Co-enrolment guidance
For parous women, participation in the trial in a previous pregnancy will be checked (as there will be a record in the electronic Source Data) in order to prevent participants from being enrolled more than once in this trial. Data of each participant should only be recorded as one entry in the database.

Screening procedures and pre-randomisation investigations
Written informed consent will be obtained from women who agree to participate in the randomised trial, after explanation of the aims, methods, benefits and potential hazards of the trial and **BEFORE** any trial-specific procedures are performed.

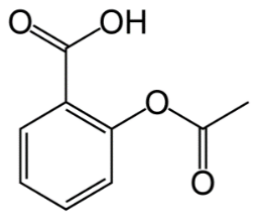
The only procedures that may be performed in advance of written informed consent being obtained are those that would be performed on all participants in the same situation as a usual standard of care, such as the routine first-trimester scan (which includes determination of chorionicity, measurement of fetal CRL and NT, assessment of fetal anatomy in both twins) and serum biochemical testing for trisomies.

We consider the point at which randomisation is complete to be the point at which a participant takes their first dose of IMP. If a participant is randomised but declines to take any IMP, we will consider the randomisation incomplete and these participants will not contribute to the target recruitment for the trial. The site must inform the coordinating centre if this occurs, who will mark the participant as being randomised in error in the randomisation system. It is not a requirement to complete a Study exit form for these participants. They will not be included in the final analysis set. The decision of whether or not to take IMP could not be influenced by knowledge of the assigned treatment.

6.4 Interventions
Women eligible to participate in this trial will provide informed consent and receive written information on the test drug. Each participant will be assigned a study ID and then be randomised to a treatment arm. The randomisation list will determine who receives placebo or investigational drug (low-dose aspirin 2 x 75mg once per day). Sealed Envelope will generate the kit code list and randomisation code list. All participants, the PI and clinical trial pharmacy, where used, will remain blind to trial drug allocation.

Intervention: The women will be asked to take two tablets of aspirin 75mg or two placebo tablets every day, ideally at night, starting on the day of randomisation and continuing until they give birth or 36 weeks' gestation (maximum duration of 174 days), whichever occurs sooner, and attend their routine schedule of antenatal scans and visits.

Active substance: 75 mg acetylsalicylic acid C9H8O4, CAS number 50-78-2), having the following molecular structure:



Comparator: Matching placebo tablets will be identical to the intervention (aspirin) in such parameters as size, thickness, physical properties and appearance.

Manufacturer: GSTT has outsourced IMP activities to RenaClinical. The aspirin and placebo will be provided by RenaClinical and released by a designated QP. They will also have responsibility for distribution of the IMP.

Packaging and labelling: 180 tablets of active (aspirin) or placebo will be packaged into a bottle with the approved trial labelling. All labelling will be compliant with the regulations. The translation from English to other relevant languages will be verified by GSTT Pharmaceuticals. Trial labels will ensure blinding of the trial medication.

QP release: RenaClinical/Eramol will release aspirin and placebo tablets packaged in bottles according to manufacturing protocol and International/European GMP. RenaClinical/Eramol will organise the packaging, labelling, storage and distribution of IMP and perform appropriate QP release.

Investigational and placebo arms

All duties described below will be undertaken either by pharmacy or the PI and delegated individuals where it is not practical to dispense directly from pharmacy. At sites where IMP is being dispensed from the ward, the clinical team will be required to complete all standard clinical trial documentation with respect to IMP management e.g. accountability logs.

Products: Enteric-coated aspirin 75 mg tablets or matching placebo tablets will be packaged into bottles (180 tablets per bottle, two bottles per participant at one dispensing event). Provision of 360 tablets in total provides each participant with 12 additional tablets to allow for possible loss of tablets.

Treatment schedule: Participants are advised to start treatment within 24 hours of randomisation.

Dispensing: Treatment packs with aspirin 75 mg tablets or placebo tablets will be dispensed according to a kit code. Individuals who dispense IMP will be blinded to who receives aspirin/placebo and the content of each bottle. There will be one dispensing event of two bottles per participant.

Dose modifications, interruptions and discontinuations: Two tablets will be administered once daily. This dosage has been carefully selected based on aspirin pharmacology and will not be changed, irrespective of indications. Participants have the right to stop taking the IMP and / or not participate in follow up visits but remain in the study. Such action will inevitably affect compliance. However, these participants are not replaced as the analysis is based on intention-to-treat. Participants have the right to withdraw consent from participating in the study at any time for any reason.

If a participant misses one or more doses of IMP in error, they should be asked to resume the standard daily dose at the earliest opportunity. They should not make up the missed doses and must not take more than 150mg daily.

If a participant wishes to withdraw from the study, this will not affect her care. All efforts will be made to report the reason for withdrawal as thoroughly as possible. These participants will not be included in the analysis of data and any of their stored samples will be destroyed.

Accountability

Trial medications will be dispensed under the supervision of a local hospital pharmacist or the clinical team. Study drugs will be supplied only to women participating in the study.

The responsible pharmacist and/or site PI and delegated staff at each clinical site, are responsible for ensuring that all study drugs at the site are inventoried and accounted for throughout the study. The dispensing of study drugs will be documented and accounted for. Study drugs will be handled in strict accordance with the protocol and will be stored in a controlled access area within the pharmacy/ward

between 15°C and 25°C. IMP will be prescribed using the appropriate trial prescription form, copies of which should be retained in the pharmacy/investigator site file. The IMP label will include a kit code.

Unused, returned or expired study drugs will be available for verification by the study personnel and the trial monitor unless authorisation to destroy IMP prior to verification from the sponsor/FMF has been received in writing prior. Before proceeding to the destruction of any IMP, authorisation from the relevant coordinating centre (FFIS or FMF) must be sought. Once authorised, the destruction will be in accordance with the Local Pharmacy Standard Operating Procedure (SOP).

Compliance and adherence

Participants will be asked to bring any unused trial medication to each trial visit; IMP compliance will be assessed by study site staff by counting remaining tablets at each follow up visit and asking about compliance at the standard telephone follow up interviews. If the participant forgets to bring the IMP during the clinical visits then an additional telephone call will be made within the subsequent three days. Compliance with other aspects of the trial protocol will also be assessed. Participants will be encouraged to report any concerns or side effects in a diary for review at each trial visit.

Concomitant care

Any other medication permitted concurrently with the study medication will be recorded in the relevant case report form (CRF) at each trial visit. This will include COVID-19 vaccination status. We advise avoiding aspirin-containing compounds and chronic use of other non-steroidal anti-inflammatory drugs. We will collect information about this in eCRF.

Overdose of trial medication

Participants will be advised to contact the designated trial doctor (telephone number will be provided at enrolment) should they have taken an overdose of the trial medication.

Protocol treatment discontinuation

In consenting to the trial, participants are consenting to trial treatments, trial follow-up and data collection. However, an individual participant may stop treatment early or be stopped early for any of the following reasons:

a. Unacceptable treatment toxicity or adverse event
b. Inter-current illness that prevents further treatment
c. Any change in the participant's condition that in the clinician's opinion justifies the discontinuation of treatment
d. Decision by the participant to stop taking the IMP
e. Withdrawal of consent for participation in the trial by the participant

As participation in the trial is entirely voluntary, the participant may choose to discontinue trial treatment at any time without penalty or loss of benefits to which they would otherwise be entitled. Although not obliged to give a reason for discontinuing their trial treatment, a reasonable effort should be made to establish this reason, whilst remaining fully respectful of the participant's rights.

Participants who discontinue protocol treatment, for any of the above reasons a, b, c and d, should remain in the trial where possible for the purpose of follow up and data analysis. These participants will be included in the intention to treat analysis of the data. Participants who wish to withdraw consent from participation in the trial (group e above) will not be included in the analysis and any of their stored samples will be destroyed.

The investigator also has the right to withdraw participants from the study drug in the event of inter-current illness, adverse events (AE), serious adverse events (SAE), suspected unexpected serious adverse reaction (SUSAR) or protocol violations. All SAEs and SUSAR will be reported to FFIS and FMF. SAEs and SUSARs will be reported to the relevant Competent Authorities according to European Commission

guidelines CT3 (2011/C, 172/01, June 2011). It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of participants should be avoided.

6.5 Outcomes

Primary Outcome

To determine if the prophylactic use of low-dose aspirin from the first-trimester of pregnancy in women with twin pregnancy can reduce the incidence of PE with delivery <37 weeks' gestation.

PE will be defined as per the American College of Obstetricians and Gynecologists (ACOG 2013). The systolic blood pressure should be ≥140 mm Hg and / or the diastolic should be ≥90 mm Hg on at least two occasions four hours apart developing ≥20 weeks' gestation in previously normotensive women (blood pressure <140/90 mm Hg) accompanied by one or more of the following new onset conditions at ≥20 weeks' gestation:

1. Proteinuria defined as ≥300mg in 24 hours or urinary creatinine ratio ≥30mg/mmol (0.3mg/mg) or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-hour collection is available.

2. Maternal organ dysfunction defined as any one of the following:
    a. acute kidney injury with creatinine >97 µmol/L (1.1 mg / dL)
    b. liver involvement with elevated transaminases (ALT or AST >90 IU/L or twice the normal concentration)
    c. hematological complications (thrombocytopenia with platelet count <100,000/µL), disseminated intravascular coagulation or hemolysis.
    d. neurological complications such as eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, persistent visual scotomata

Secondary Outcomes

To determine the effect of low-dose aspirin on the incidence of (stratified according to chorionicity) :

- Delivery with PE at <32 weeks, <34 weeks, <37 weeks and at any gestation.
- Features of severe PE including:
    - stroke
    - Eclamspia
    - systolic blood pressure >160 mmHg on at least one occasion
    - diastolic blood pressure >110 mmHg on at least one occasion
    - respiratory failure requiring intubation or mechanical ventilation
    - myocardial ischemia or infarction
    - pulmonary edema
    - hepatic dysfunction (INR >1.2 in the absence of DIC)
    - hepatic hematoma or rapture
    - platelet count <100 x 109/litre
    - abnormal liver function enzymes (ALT or AST >67 iu/litre)
    - acute kidney injury
    - creatinine >150 µmol/L
    - cortical blindness
    - retinal detachment
    - transfusion of any blood products
    - HELLP syndrome
    - placental abruption
    - postpartum hemorrhage (defined as blood loss ≥1 L within the first 24 hours after birth)

- intensive therapy or high-dependency unit admission
- intubation and mechanical ventilation
- confirmed sepsis (positive blood or urine cultures) up to post-natal discharge
- total number of nights in hospital

- Gestational hypertension (GH)
- Birth at <32 weeks, <34 weeks and <37 weeks
  - Spontaneous
  - Iatrogenic for PE, GH or FGR
  - Iatrogenic for other reason
- Death of one twin and / or both twins before discharge from hospital
  - Miscarriage of the whole pregnancy or death of one twin <24 weeks' gestation.
  - Stillbirth or neonatal death of one or both twins at <32 weeks, <34 weeks, <37 weeks and at any gestation.
- Birthweight <3rd, <5th and <10th percentile for gestational age.
- Placental abruption (clinically or on placental examination) at <32 weeks, <34 weeks, <37 weeks and at any gestation.
- Postpartum hemorrhage (defined as blood loss ≥1 L within the first 24 hours after birth).

- Neonatal morbidity
  - Intraventricular hemorrhage (IVH) grade II or above – Defined as bleeding into the ventricles
    - Grade II (moderate) – IVH occupies <50% of the lateral ventricle volume
    - Grade III (severe) – IVH occupies ≥50% of the lateral ventricle volume
    - Grade IV (severe) – Hemorrhagic infarction in periventricular white matter ipsilateral to a large IVH
  - Neonatal sepsis confirmed bacteremia in cultures
  - Encephalopathy grade (mild, moderate, severe)
  - Neonatal seizures
  - Anemia defined as low hemoglobin and / or hematocrit requiring blood transfusion
  - Respiratory distress syndrome defined as need of ventilation with or without surfactant
  - Necrotizing enterocolitis requiring surgical intervention
  - Composite of any of the above
- Neonatal therapy
  - Neonatal intensive care unit admission
  - Ventilation defined as need of positive pressure (continuous positive airway pressure (CPAP) or nasal continuous positive airway pressure (NCPAP)) or intubation
  - Composite of any of the above
- Length of stay in neonatal intensive care unit

Birth weight percentile for gestational age at delivery will be calculated using the Fetal Medicine Foundation birthweight chart (Nicolaides et al., 2019).

GH will be defined as per the American College of Obstetricians and Gynecologists (ACOG 2013). The systolic blood pressure should be ≥140 mm Hg and / or the diastolic should be ≥90 mm Hg on at least two occasions four hours apart developing ≥20 weeks' gestation in previously normotensive women (blood pressure <140/90 mm Hg) in the absence of proteinuria or maternal organ dysfunction.

Collection of pregnancy and neonatal outcomes

Data on pregnancy outcome will be collected from hospital maternity records or general practitioners. The obstetric records of participants with pre-existing or pregnancy-associated hypertension will be examined to determine if the condition was chronic hypertension, PE or GH.

In the event when the neonates are admitted to Special Care Baby Unit (SCBU) / Neonatal Intensive Care

Unit (NICU), additional neonatal outcomes will be collected from the discharge summary of SCBU / NICU.

Other secondary efficacy parameters will be assessed by the clinical team at the time of delivery and recorded in the participants' medical notes (all data is routine clinical practice) and will be transcribed into the trial CRF by the trial team.

6.6 Participant Timeline:

The trial procedures by visit

Routine screening visit (11^{+2}-13^{+6} week of pregnancy or CRL 45-84 mm)

Recording of: demographic characteristics, medical and obstetric history and concomitant medications (including COVID-19 vaccination status)

- Measurements of: height, weight, blood pressure and UtA-PI according to FMF guidelines
- Ultrasound scan for: measurement of CRL and NT and examination of each fetus for major defects
- Pregnancy dating: from CRL of biggest twin
- Blood sample for storage for future research (only in centres with available facilities)
- Patients meeting eligibility criteria and agreeing to participate in the trial: Informed consent, randomisation, IMP dispensing and taking of the first tablet either at this visit or (for those that require further time to consider their options) in a subsequent randomization visit up to 14^{+3} weeks

Randomisation visit (11^{+2}-14^{+3} week of pregnancy)

- Informed consent, randomisation, IMP dispensing and taking of the first tablet
- This visit could be the same as the routine screening visit above

Follow up visits

For the purpose of the trial the following are the necessary visits (in clinical management of participants there will be additional visits and additional measurements as per local hospital protocol): 20^{+0}-21^{+6}, 28^{+0}-29^{+6}, 31^{+0}-32^{+6}, 35^{+0}-36^{+6} weeks' gestation.

- Blood pressure at each visit. If ≥140/90 then routine tests (creatinine, liver enzymes, platelets, urinalysis for protein)
- Uterine artery PI at each visit depending on capacity
- Routine ultrasound examination for fetal anatomy and growth, placental localization (low, previa or high) and amniotic fluid volume (deepest pool for each sac)
- Doppler measurements of umbilical artery PI, middle cerebral artery PI and ductus venosus PI (optional)
- Blood taking for storage for future research at 20 and 32 weeks (only in centres with available capacity)
- Recording of: concomitant medication (including COVID-19 vaccination status), adverse events / side effects and IMP compliance
- Stop IMP at 36 weeks

Telephone interviews

For the purpose of the trial the following are the necessary telephone interviews: 15^{+0}-16^{+6}, 24^{+0}-25^{+6} and 30 days after the last dose of IMP.

- Recording of: concomitant medication (including COVID-19 vaccination status), adverse events / side effects and reminding participants to take their IMP

The period for adverse events reporting will be from the time of the first dose until 30 days after the last dose of IMP.

When the participant completes the trial, the participant status will be collected on a follow up form (part of the CRF). If the participant has a miscarriage or has delivered before 36 weeks, the trial co-ordinators will contact these women and ask them to return the trial medication separately.

Early stopping of follow-up

If a participant chooses to discontinue their trial treatment, they should continue to be followed up as closely as possible to the follow-up schedule defined in the protocol, providing they are willing. They should be encouraged and facilitated not to leave the whole trial, even though they no longer take the trial treatment and / or do not wish to attend the follow up visits. Their data will be kept and included in analyses according to the intention-to-treat principle.

If the participant exercises the view that she wants to withdraw consent from participating in the study her decision must be respected. These participants will not be included in the analysis of data and any of their stored samples will be destroyed. The FMF, for sites in the UK, Ireland, Germany, Belgium, Denmark, Bulgaria, Greece, Hungary, Portugal, Poland, Italy, Austria, Australia, Argentina, Brazil and Israel or FFIS for sites in Spain, should be informed of the withdrawal in writing using the appropriate trial documentation.

Participant transfers

If a participant moves from the area making continued follow up at their consenting centre inappropriate, every effort should be made for them to be followed at another participating trial centre. Written consent should be taken at the new centre and then a copy of the participant's CRFs should be provided to the new centre. Responsibility for the participant remains with the original consenting centre until the new consent process is complete.

Loss to follow-up

For participants that are lost to follow up, every effort should be made to contact their general practitioners in order to acquire the pregnancy outcomes.

Trial closure

The end of the study for individual participants will be defined as 30 days after the birth of the babies / end of the pregnancy. The end of the study as a whole will be defined as the last visit of the last participant with details of their complete pregnancy outcome.

Competent authorities in the member states concerned will be notified of the end of the trial within 90 days of its completion.

6.7 Sample size

The sample-size calculation presented here is based on detecting a treatment effect that produces a 50% reduction in the incidence of preterm PE from an anticipated 5.5% in the placebo group to 2.75% in the aspirin group. A total sample of 2,200 participants has 90% power of detecting this difference at a (two-tailed) significance level of 5%. Allowing for 9% attrition, it will be necessary to randomise a total of 2,400 pregnancies. On the assumption that 60% of women with twin pregnancies fulfilling the entry criteria agree to participate in the study, we would need to approach 4,000 such women.

6.8 Recruitment and retention

Recruitment

In all the participating centres, all eligible women attending for their first-trimester scan will be invited to take part in the randomised controlled trial by the designated trial teams. Recruitment rates will be actively monitored by the TMG. This will include analyses by centre of the number of women invited to participate in the trial and the number randomised. Appropriate strategies will be implemented if recruitment falls below an acceptable level.

Retention

If women fail to attend their follow-up visits, the trial coordinators will contact them to arrange for another visit within 7 days.

COVID-19 Impact
The impact of COVID-19 on recruitment and retention to the trial has been considered and we believe the impact will be minimal because all on-site trial visits coincide with routine clinical visits. Should a participant test positive for COVID-19 or display symptoms consistent with a COVID-19 infection, local hospital policy on attendance at the site will be followed.

6.9 Assignment of intervention

Sequence generation: Randomisation will be by random permuted blocks and participants will be stratified by centre and chorionicity. The treatment allocation will only be revealed to the researchers after completion of the study or where clinically essential.

Allocation concealment mechanism: Each treatment pack will only be identified by a kit code. When a participant enrols onto the study, the randomisation system will assign participants two kit codes corresponding to the treatment arm to which they have been assigned. If IMP is being dispensed from a pharmacy, the participant or a member of the clinical team, on behalf of the participant, will bring the prescription with the allocated kit codes detailed to the pharmacy, where the pharmacy will provide her with the bottles matched to the kit codes. Neither the pharmacy at each clinical site or the clinical staff will have access to the codes, except in case of an emergency.

Allocation Implementation: Responsibility for who is enrolled in the trial and receives IMP is the Principal Investigator's (PI). S/he makes his/her eligibility decision according to the approved protocol. Other physicians, employed at the same Clinical Site may enrol participants as long as they appear on the Trial Delegation log and are signed off by the PI.

Blinding
RenaClinical will provide labelling for the IMP bottles ensuring complete blinding of the IMP to all investigators and participants in the study. That includes the PI, participating research doctors, pharmacists at the local clinical trial pharmacy, project managers and others involved in the trial. They are all blinded to the allocation. As mentioned previously, the tablets will be identical, so it will not be possible to distinguish between the active (aspirin) and placebo. RenaClinical and Sealed Envelope will keep the kit codes confidential to maintain the blind.

Emergency unblinding
In case of emergency, the facility to unblind a participant's treatment allocation will be available at all times during the study via an online service provided by Sealed Envelope, which has a telephone unblinding backup system in cases where the hospital network is not functioning. The coordinating team will receive an email informing them a participant has been unblinded but this email will not reveal the treatment allocation.

6.10 Data collection, management and analysis

Data collection
All of the main participant information for this study will be entered into an electronic CRF that will be printed and signed by the enrolling researcher. The CRF will be composed of 3 parts:

CRF part 1 This will include participant demographics, medical and obstetric history, measurements of MAP and UtA-PI, performance of an ultrasound scan to confirm the gestational age by the measurement

of fetal CRL of the bigger twin, eligibility criteria, and randomization details. Participants will be identified on CRFs by participant code and site name, date of enrolment and the enrolling site-PI or fellow.

CRF part 2 This will record details of the routine clinical visits (including ultrasound findings, MAP, IMP compliance and adverse events) and details of the telephone interviews.

CRF part 3 This will record details of pregnancy and postnatal outcomes.

All parts of CRFs should be signed by the site-PI and / or fellows for each participant enrolled, including those removed / withdrawn from the study for any reason. The reason for removal/withdrawal must be noted on the study conclusion CRF by the site-PI. CRFs must be kept current to reflect the participant's status at each phase during the course of the study.

The CRFs will be the source documents of the study that must be available at all times for inspection by the regulatory authorities. A participant identification record will be kept by each site-PI that would allow linking of the participant study number, participant name and date of birth for those included in the study along with participant contact information. This file will be kept electronically at sites.

Ancillary CRFs

Ancillary CRFs, including exceptions to SAEs, SAEs and study exit forms will be available within the Sealed Envelope/Red Pill system. These should be completed, as required. If an SAE form is completed, the participating site must also email FFIS/FMF immediately following completion of the eCRF to alert them that an SAE form has been submitted so that a clinical review can be completed.

Non-adherence and non-retention

For non-adherers (as defined by poor attendance to clinical visits) we will record details of pregnancy and postnatal outcomes as for adherers. Reasons for non-adherence and non-retention and those lost to follow up will be recorded in the CRF.

Data management

The Chief Investigators will act as custodian for the trial data. The following guidelines will be strictly adhered to:

- Participant data will be pseudo-anonymised.
- All pseudo-anonymised data will be stored on GDPR-compliant servers and/or a password-protected computer.

Statistical methods

Statistical analysis plan: A stand-alone Statistical Analysis Plan (SAP) detailing the analysis will be produced before follow up is complete. To ensure that the analysis can be validated and is reproducible, this document will include listings of all program code used for the statistical analysis together with sample outputs, tables and figures. The SAP may be informed by analyses of data blinded to treatment, but it will be finalised before the blind is broken. The SAP will be approved by the IDMC and by the TSC.

Results will be reported according to the CONSORT statement (Moher et al., 2001). It is envisaged that the analysis will be undertaken using R and WinBugs software.

Summary statistics will be produced for aspirin and placebo groups. Continuous data will be summarized in terms of means, standard deviations, minimum, maximum and quartiles. Attribute data will be summarised in terms of frequency counts and proportions.

Statistical analyses will be performed on an intention-to-treat basis. Logistic regression analysis will be used to determine the significance of difference in incidence of preterm-PE between the aspirin and placebo

groups, adjusting for the effect of centre (random effect) and the logistic transformation of risk. The treatment effect will be quantified as odds ratio with 95% confidence interval (CI) in the aspirin group. Homogeneity of the treatment effect will be examined by centre, chorionicity, risk group and specific maternal characteristics. Sensitivity analysis will be performed to examine the effect of withdrawal of consent and loss to follow up. Kaplan-Meier estimates of the cumulative incidence of PE by treatment group, in which deliveries without PE are treated as censored observations, will be produced. The statistical software package R will be used for data analysis. Results will be presented as 95% confidence intervals with no corrections for multiple testing.

Safety: The incidence rates of adverse events and serious adverse events and their relationship to trial drugs will be summarised by treatment group. The proportion of women discontinuing treatment will be summarised by reason and by treatment group. All investigators, participants and clinicians will be unaware of the treatment groups. All outcomes will be determined before the blind of the trial is broken.

6.11 Data monitoring

Independent Data Monitoring Committee

The Independent Data Monitoring Committee (IDMC) is independent of the trial and is responsible for monitoring the progress of the trial including: recruitment, protocol adherence, serious adverse events and side effects of treatment as well as the difference between the trial treatments on the primary outcome measures. The IDMC will be appointed and will meet at least annually to assess the safety data. They will provide a confidential trial progress report at the end of each meeting, which will be sent to the TSC. The chief investigator (or their representative), trial statistician and other trial staff may be in attendance for the open session of the IDMC meeting. Further details of the roles and responsibilities of the IDMC, including membership, relationships with other committees, decision-making processes, and the timing and frequency of interim analyses (and description of stopping rules and/or guidelines where applicable) are described in detail in the IDMC Terms of Reference.

Interim analyses

Given that recruitment will be completed before outcome data are available on a sufficient number of participants, no interim analyses are scheduled. However, depending on factors such as the recruitment rate, the IDMC may decide on the need for an interim analysis.

Data monitoring for harm: safety of aspirin

Any unfavourable and intended sign, symptom or illness that develops or worsens during the period of the study will be classified as an adverse event (AE), whether or not it is considered to be related to the study treatment. Adverse events will include unwanted side effects, sensitivity reactions, abnormal laboratory results, injury or inter-current illnesses, and may be expected or unexpected. These will be recorded electronically on the CRF.

Aspirin at high doses may induce hypersensitivity and asthma, may cause urate kidney stones, chronic gastro-intestinal blood loss, tinnitus, nausea and vomiting, and has been reported (with high blood salicylate levels) to prolong pregnancy and labour, with increased bleeding before and after delivery, decreased birth weight and increased rate of stillbirth. However, several major randomised controlled trials using low-dose aspirin have shown no adverse effects to the mother or the fetus during pregnancy, delivery or epidural anaesthesia. The Collaborative Perinatal Project prospectively monitored 50,282 mother-child pairs, 64% of which were exposed to aspirin at some point during pregnancy; it found no differences in outcomes between those exposed and those not exposed to aspirin. Furthermore, longer-term follow up studies are now emerging which attest to the safety of aspirin on the development of children exposed to aspirin in utero. In an exhaustive literature review on the use of low dose aspirin in pregnancy, Dekker and Sibai (1993) conclude that ‘there is no evidence that low-dose aspirin carries any significant maternal or fetal risks’.

Safety evaluations will be conducted in each follow-up visit and the Co-PI or site-PIs can be directly contacted by the participants if there are any concerns regarding their medication. The period for adverse event reporting will be from the time of complete randomisation (i.e. the first IMP administration) until 30 days post final IMP administration. The participants will be followed up by a telephone interview 30 days after the last dose of IMP. All events will be followed until resolution if that means beyond the timelines defined here.

Safety reporting: Definitions of harm of the EU Directive 2001/20/EC Article 2 based on the principles of ICH GCP apply to this trial.

Table 1: Adverse Event Definitions

Adverse event (AE)	Any untoward medical occurrence in a participant or clinical trial participant administered a medicinal product and which does not necessarily have a causal relationship with this product.
Adverse reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
Unexpected adverse reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the applicable product information (eg Investigator's Brochure for an unauthorised product or summary of product characteristics (SPC) for an authorised product.
Serious adverse event (SAE) or serious adverse reaction (SAR)	Any AE or AR that at any dose: • results in death • is life threatening* • requires hospitalisation or prolongs existing hospitalisation** • results in persistent or significant disability or incapacity • is a congenital anomaly or birth defect • or is another important medical condition***
* the term life threatening here refers to an event in which the participant is at risk of death at the time of the event; it does not refer to an event that might hypothetically cause death if it was more severe (eg a silent myocardial infarction) ** Hospitalisation is defined as an in-patient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisation for pre-existing conditions (including elective procedures that have not worsened) do not constitute an SAE *** Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. Important AEs or ARs that may not be immediately life threatening or result in death or hospitalisation, but may seriously jeopardise the participant by requiring intervention to prevent one of the other outcomes listed in the table (eg. A secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not require hospitalisation, or development of drug dependency).	

Adverse events include:

- Exacerbation of a pre-existing illness
- Increase in the frequency or intensity of a pre-existing episodic event or condition
- A condition (regardless of whether PRESENT prior to the start of the trial) that is DETECTED after trial drug administration. (This does not include pre-existing conditions recorded as such at baseline – as they are not detected after trial drug administration.)
- Continuous persistent disease or a symptom present at baseline that worsens following administration of the trial treatment

Adverse events do NOT include:

- Medical or surgical procedures: the condition that leads to the procedure is the adverse event
- Pre-existing disease or a condition present before treatment that does not worsen
- Hospitalisation where no untoward or unintended response has occurred eg. elective cosmetic surgery
- Overdose of medication without signs or symptoms

Investigator responsibilities relating to safety reporting: FFIS will act as sponsor for the study and has delegated the delivery of the Sponsor's responsibility for Pharmacovigilance, (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004, to the FMF for informing ethics committees in the sites coordinated by the FMF. FFIS retain responsibility for reporting to competent authorities and preparation of development safety update reports. Sites in Australia, Argentina, Brazil and Israel will report to their local Sponsors and the FMF/FFIS. All non-serious AEs and ARs, whether expected or not, should be recorded in the participant's medical notes and recorded in the main eCRF. All protocol defined exceptions to SAEs must be recorded in the main eCRF if they are a trial outcome and in the ancillary CRF if they are not a trial outcome but fall into the definition of a SAE which does not require expedited reporting to the sponsor below.

Protocol defined exceptions to SAE reporting: Events which **do not require expedited** reporting by sites to their sponsor and coordinating centre are defined as follows:

Trial Outcome SAE Exceptions:

Any event which fulfils the definition of an SAE but is **NOT** considered to be related to the IMP and is captured in the **main trial outcome eCRF**. This includes, but is not limited to: hypertension, preeclampsia, preterm delivery, preterm premature rupture of membrane, miscarriage, stillbirth or neonatal death, admission of the baby to NICU and termination of one or more fetus for fetal or maternal indication. Please ensure the indication for an admission/procedure is recorded.

Other SAE Exceptions:

Other events which fulfil the definition of an SAE and are exempt from expedited reporting but are not captured in the main trial outcome eCRF must fulfil **ALL** of the following criteria:

- NOT considered to be related to the IMP
- Must be considered related to the routine course of the individual pregnancy as assessed at inclusion in the trial

Examples include, but are not limited to, admission for observation, admission for procedures which could be expected at inclusion in the trial e.g. cervical cerclage, twin-twin transfusion syndrome. Please ensure the indication for an admission/procedure is recorded.

Admission for delivery and caesarean section is an additional exception to SAE reporting that does not require reporting or recording as an SAE or exception. If appropriate, the cause of an admission for delivery/ caesarean section will be recorded.

These events are exempt from expedited reporting to the sponsor/coordinating centre but a summary of these events will be included in IDMC reports/DSURs.

Seriousness assessment: When an AE or AR occurs, the investigator responsible for the care of the participant must first assess whether or not the event is serious using the definition given in Table 1. If the event is classified as 'serious' and is not an exception, then an SAE form must be completed within the Sealed Envelope/Red Pill system and the FFIS and FMF notified that a form has been completed within 24 hours.

Severity or grading of adverse events: The severity of all protocol defined exceptions to SAE reporting and SAEs/SARs in this trial should be graded using the toxicity grading according to the Common Toxicity Criteria (version 5, 27 November 2017): (1) Mild, (2) Moderate, (3) Severe, (4) Life threatening, (5) Death.

Causality: The investigator must assess the causality of all serious events or reactions in relation to the trial therapy using the definitions in Table 2. If an SAE is considered to be related to trial treatment, and treatment is discontinued, interrupted or the dose modified, refer to the relevant Interventions sections of the protocol.

Table 2: Causality definitions

Relationship	Description	Event type
Unrelated	There is no evidence of any causal relationship	Unrelated SAE
Unlikely to be related	There is little evidence to suggest that there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition or other concomitant treatment)	Unrelated SAE
Possibly related	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition or other concomitant treatment)	SAR
Probably related	There is evidence to suggest a causal relationship and the influence of other factors is unlikely	SAR
Definitely related	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	SAR

Expectedness: If there is at least a possible involvement of the trial medications (including any comparators), the sponsor must assess the expectedness of the event. An unexpected adverse reaction is one that is not reported in the current SPCs, or one that is more frequently reported or more severe than previously reported. See section 4.8 of SPC for a list of expected toxicities associated with the drugs being used in this trial. If a SAR is assessed as being unexpected it becomes a SUSAR (suspected, unexpected, serious adverse reaction) and the relevant competent authorities and REC reporting guidelines apply (see Notifications sections of the protocol).

Notifications

Notifications by the investigator to the sponsor: The FFIS and FMF must be notified of all SAEs which require expedited reporting within 24 hours of the investigator becoming aware of the event. Investigators should notify the FFIS and FMF of any SAEs occurring from the time of complete randomisation until 30 days after the last dose of IMP. SARs and SUSARs must be notified to the FFIS and FMF until trial closure.

The SAE form must be completed by the investigator within Sealed Envelope/Red Pill (the consultant named on the delegation of responsibilities list who is responsible for the participant's care) with attention paid to the grading and causality of the event. An email must also be sent to FFIS/FMF to alert them to the submission of an SAE form to ensure a clinical review is completed. In the absence of the responsible investigator, the SAE form should be completed and signed by a member of the site trial team and emailed as appropriate within the timeline. The responsible investigator should check the SAE form at the earliest opportunity, make any changes necessary. Detailed written reports should be completed as appropriate. Systems will be in place at the site to enable the investigator to check the form for clinical accuracy as soon as possible. The minimum criteria required for reporting an SAE are the study ID and date of birth,

name of reporting investigator and sufficient information on the event to confirm seriousness. Any further information regarding the event that is unavailable at the time of the first report should be sent as soon as it becomes available.

Participants must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline values, or until the event has stabilised. Follow-up should continue after completion of protocol treatment and/or trial follow-up if necessary. Follow-up SAE forms (clearly marked as follow-up) should be completed within Sealed Envelope/Red Pill and an email sent to FFIS/FMF to alert them it has been completed as further information becomes available. Additional information and/or copies of test results etc. may also be provided. The participant must be identified by study ID, date of birth only. The participant's name should not be used on any correspondence and should be blacked out and replaced with trial identifiers on any test results.

The FMF and FFIS responsibilities: Medically qualified staff at the FMF and FFIS and/or the Co-Chief Investigators (Co-CIs or a medically qualified delegate) will review all SAE reports received. In the event of disagreement between the causality assessment given by the local investigator and the CI, both opinions and any justifications will be provided in subsequent reports. The delegated staff at the FMF and FFIS will review the assessment of expectedness. FFIS and FMF will ensure SAEs arising at sites coordinated by them are reviewed and logged and reported to relevant ethics committees. FFIS remains responsible for the reporting of SUSARs and other SARs to all the regulatory authorities (e.g. AEMPS in Spain). Fatal and life threatening SUSARs must be reported to the competent authorities within seven days of the FFIS becoming aware of the event; other SUSARs must be reported within 15 days. The FMF and FFIS will keep investigators informed of any safety issues that arise during the course of the trial. The sponsor will submit Development Safety Update Reports (DSURs) to competent authorities. Sites in Australia, Argentina, Brazil and Israel will report to their local Sponsors, ethics committees and competent authorities.

Quality assurance and control

Risk assessment
The Quality Assurance (QA) and Quality Control (QC) considerations for the ASPRE-T trial includes a formal Risk Assessment, and that acknowledges the risks associated with the conduct of the trial and proposals of how to mitigate them through appropriate QA and QC processes. Risks are defined in terms of their impact on: the rights and safety of participants; project concept including trial design, reliability of results and institutional risk; project management; and other considerations.

QA is defined as all the planned and systematic actions established to ensure the trial is performed and data generated, documented and/or recorded and reported in compliance with the principles of GCP and applicable regulatory requirements. QC is defined as the operational techniques and activities performed within the QA system to verify that the requirements for quality of the trial related activities are fulfilled.

Central monitoring at the FMF and the FFIS
The staff at the FMF and FFIS will review Case Report Form (CRF) data for errors and missing key data points at regular intervals. Those entries will be reviewed by the researchers at the recruiting centres and amended accordingly.

On-site monitoring
The frequency, type and intensity of routine and triggered on-site monitoring will be detailed in the Quality Management Plan (QMP). The QMP will also detail the procedures for review and sign- off of monitoring reports. Participating investigators must agree to allow trial related monitoring, including audits, REC review and regulatory inspections, by providing access to source data and other trial related documentation as required. Participant consent for this must be obtained as part of the informed consent process for the trial. Source data location lists will be prepared for all each site.

Trial oversight

Trial oversight is intended to preserve the integrity of the trial by independently verifying a variety of processes and prompting corrective action where necessary. The processes reviewed relate to participant enrolment, consent, eligibility, and allocation to trial groups; adherence to trial interventions and policies to protect participants, including reporting of harms; completeness, accuracy and timeliness of data collection; and will verify adherence to applicable policies detailed in the Compliance section of the protocol. In multi-centre trials this oversight is considered and described both overall and for each recruiting centre by exploring the trial dataset or performing site visits as described in the Quality Management and Monitoring Plan.

Trial Management Group: A Trial Management Group (TMG) will be set up to assist with developing the design, co-ordination, strategic management of the trial and day-to-day operational issues, including budget management. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the TMG terms of reference.

Trial Steering Committee: The Trial Steering Committee (TSC) is the group responsible for oversight of the trial in order to safeguard the interests of trial participants. The TSC provides advice to the CI, Co-CIs, the funder and Sponsor on all aspects of the trial through its independent Chair. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the TSC terms of reference.

Independent Data Monitoring Committee: The Independent Data Monitoring Committee (IDMC) is the only oversight body that has access to unblinded accumulating comparative data. The IDMC is responsible for safeguarding the interests of trial participants, monitoring the accumulating data and making recommendations to the TSC on whether the trial should continue as planned. The membership, frequency of meetings, activity (including review of trial conduct and data) and authority will be covered in the IDMC terms of reference. The IDMC will consider data in accordance with the statistical analysis plan and will advise the TSC through its Chair.

7. ETHICS AND DISSEMINATION

Research ethics approval

This protocol and related documents will be submitted for review to REC and relevant competent authorities for Clinical Trial Authorisation. Where required, annual progress and safety reports, and a final report at the conclusion of the trial, will be submitted to the RECs and relevant competent authorities within the timelines defined in the Regulations.

Before initiation of the trial at any clinical site, the protocol, all informed consent forms and any material to be given to the prospective participant will be submitted to the relevant REC for approval. Any subsequent amendments to these documents will be submitted for further approval. Before initiation of the trial at each additional clinical site, the same/amended documents will be submitted for local Research and Development (R&D) approval.

The rights of the participant to refuse to participate in the trial without giving a reason must be respected. After the participant has entered the trial, the clinician remains free to give alternative treatment to that specified in the protocol, at any stage, if s/he feels it to be in the best interest of the participant. The reasons for doing so must be recorded. After randomisation the participant must remain within the trial for the purpose of follow up and data analysis according to the treatment option to which they have been allocated. However, the participant remains free to change their mind at any time about the protocol treatment and follow-up without giving a reason and without prejudicing their further treatment.

Competent authority approvals

This protocol will be submitted to the national competent or equivalent authority (e.g. AEMPS), as appropriate in each country where the trial will be conducted. This is a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC. Therefore, a CTA is required in the member states concerned.

The progress of the trial, safety issues and reports, including expedited reporting of SUSARs, will be reported to the Competent Authority, regulatory agency or equivalent in accordance with relevant national and local requirements and practices.

Other approvals

The protocol will be submitted by those delegated to do so to the relevant R&D department of each participating site or to other local departments for approval as required in each country. A copy of the local approval (or other relevant approval as above) and of the Participant Information Sheet (PIS) and consent form on local headed paper must be forwarded to the co-ordinating centre before participants are randomised to the trial.

Protocol amendments

Substantial protocol amendments (e.g. changes to eligibility criteria, outcomes, sample size calculations, analyses) will be decided by the Co-Chief Investigators. Such amendments will be submitted to REC and competent authorities for approval. Once approved, each site PI will be notified via email.

Consent

During the consent process it will be made completely and unambiguously clear that the participant is free to refuse to participate in all or any aspect of the trial, at any time and for any reason, without incurring any penalty or affecting their treatment.

Consent will be re-sought if new information becomes available that affects the participant's consent in any way. This will be documented in a revision to the participant information sheet and the participant will be asked to sign an updated consent form. These will be approved by the ethics committee prior to their use.

Consent in ancillary studies: Informed consent will be sought from participants for their remaining serum and plasma (following analysis of PAPP-A and free β-hCG for risk assessments of aneuploidies) to be stored at -80^{o}C for future studies of potential biochemical markers for pregnancy complications. In addition, serum and plasma samples will be collected at 20^{+0}-21^{+6}, 31^{+0}-32^{+6} weeks' gestation for storage for use in future research on pregnancy complications in sites with such capacity.

Confidentiality

Record of participants' demographic data, ultrasound scan and clinical findings and observations and biochemical data are routinely stored in one of the two commonly used password secured data management programmes in Obstetrics (astraia Obstetrics [astraia software gmbh, Munich, Germany] or ViewPoint [GE Healthcare gmbh, Solingen, Germany]). Participants will then be only identified by their participant codes. Access is limited to authorised trial personnel (Co-CIs, site-PIs, database managers and statisticians). They can only access the data with a password. This approach of data collection will enable the collection of the complete participant records while maintaining confidentiality, so as to comply fully with the blanket requirement for anonymity of data.

Declaration of interests

The investigators named on the protocol have no financial or other competing interests that impact on their responsibilities towards the scientific value or potential publishing activities associated with the trial.

Archiving

The investigators agree to archive and/or arrange for secure storage of local ASPRE-T trial materials and records for a minimum of 5 years after the close of the trial unless otherwise advised by the Sponsor.

Access to data

Requests for access to trial data will be considered, and approved in writing where appropriate, after formal application to the TMG/TSC. Considerations for approving access are documented in the TMG/TSC Terms of Reference.

Ancillary and post-trial care

Not available

Publication policy

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. The results of the trial will be disseminated regardless of the direction of effect. The study protocol will be published on the Fetal Medicine Foundation website (www.fetalmedicine.org).

8. ANCILLARY STUDIES

It is intended that the data collected within the remit of this study shall be used to formulate models to screen for pregnancy complications occurring in multiple gestations including preterm birth, obstetric cholestasis, gestational diabetes, TTTS and selective fetal growth restriction.

9. PROTOCOL AMENDMENTS

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