# PROTOCOL FULL TITLE:

**HYPATIA: A prospective randomised controlled trial of HYdroxychloroquine to improve Pregnancy outcome in women with AnTIphospholipid Antibodies**

## Trial Identifiers

EudraCT Number – 2016-002256-25

IRAS Number – 170254

## Sponsor

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# 1. Study Synopsis

| | |
|---|---|
| Title of clinical trial | **HYPATIA: A prospective randomised controlled trial of HYdroxychloroquine to improve Pregnancy outcome in women with AnTIphospholipid Antibodies** |
| Protocol Short Title/Acronym | **HYPATIA: A study of HYdroxychloroquine to improve Pregnancy outcome in women with AnTIphospholipid Antibodies** |
| Trial Phase if not mentioned in title | III |
| Sponsor name | Rigshospitalet, Copenhagen, Denmark |
| Chief Investigator | Prof Søren Jacobsen |
| Eudract number | 2016-002256-25 |
| REC number | TBC |
| IRAS Number (UK) | 170254 |
| Medical condition or disease under investigation | Pregnant women with antiphospholipid antibodies. |
| Purpose of clinical trial | Our aim is to conduct a randomized controlled trial of hydroxychloroquine (HCQ) versus placebo in women planning to fall pregnant with underlying antiphospholipid antibodies assessing their pregnancy outcome. |
| Primary objective | To study the effect of HCQ on pregnancy outcome in women with antiphospholipid antibodies. |
| Secondary objective | To collect blood samples for pharmacokinetic and biomarkers studies |
| Trial Design | A randomized controlled trial of HCQ versus placebo supplementing usual medication during pregnancy in women with antiphospholipid antibodies (aPL). |
| Endpoints | The primary endpoint is a composite of three principal aPL-related adverse pregnancy outcomes: one or more pregnancy loss(es) (either < 10 weeks gestation or beyond 10 weeks of gestation of a morphologically normal fetus documented by ultrasound or by direct examination of the fetus) and premature birth of a morphologically normal neonate before 34 weeks due to any of: pre-eclampsia, eclampsia, recognized features of placental insufficiency |

| | |
|---|---|
| | Premature birth for other reasons will not be included.<br><br>The components of the primary endpoint will each be presented as secondary endpoints (below).<br><br>The pre-defined secondary endpoints include:<br>1. Pregnancy loss < 10 weeks gestation<br>2. Pregnancy loss > 10th week of gestation of a morphologically normal fetus documented by ultrasound or by direct examination of the fetus<br>3. Premature birth of a morphologically normal neonate < 34 weeks due to any of: pre-eclampsia, eclampsia, recognized features of placental insufficiency.<br>4. Gestational age at delivery<br>5. Birth weight<br>6. Delivery by Caesarean section<br>7. Apgar score < 7 at 5 min<br>8. Neonatal morbidity (bleeding or thrombotic complications, infections, congenital abnormalities)<br>9. Days to hospital discharge following delivery (mother & child)<br>10. Thrombotic events in the mother during pregnancy and 6 weeks post-partum.<br>11. Days of neonate in special care<br>12. Safety and tolerability of hydroxychloroquine in the mother and in the neonate |
| Sample Size | 328 pregnant women (in order to achieve this number we will randomize 400 patients) |
| Summary of eligibility criteria | Inclusion criteria:<br>1. Women with known aPL (i.e. isolated aPL or APS) who are planning pregnancy.<br>aPL are defined by the presence of a positive test for anticardiolipin antibodies (IgG/IgM isotypes > 95th percentile) and/or lupus anticoagulant and/or anti- beta 2 glycoprotein-I (IgG/IgM isotypes > 95th percentile), on two or more consecutive occasions more than 12 weeks apart (a positive aPL test is defined under 'glossary and definitions'). The last positive test must be within 12 months of study entry.<br>2. Written informed consent to participate<br><br>Exclusion criteria:<br>1. Women who are already pregnant<br>2. Allergy or adverse event to hydroxychloroquine. Hypersensitivity to the active substance, 4-aminoquinoline or any of the compounds of the IMP or placebo.<br>3. Current treatment with hydroxychloroquine |

| | |
|---|---|
| | 4. Age < 18 years and > 45 years<br>5. Body weight < 45 kg<br>6. Psoriasis<br>7. Uncontrolled epilepsy<br>8. Anti-Ro antibodies<br>9. Renal replacement therapy<br>10. Other severe active co-morbidities (HIV, hepatitis B, severe gastrointestinal, neurological or blood disorders)<br>11. Porphyria<br>12. History of retinopathy or newly diagnosed retinopathy<br>13. History of galactose intolerance, lactase deficiency or glucose-galactose malabsorption<br>14. History of glucose-6-dehydrogenase deficiency<br>15. Participation in any other IMP trial at the time of consent<br>16. Previous pregnancy failure on hydroxychloroquine |
| IMP, dosage and route of administration | **Treatment arm:** Oral hydroxychloroquine 200 mg once daily in addition to usual care and medication starting from trial entry and throughout pregnancy until day of delivery.<br><br>**Placebo arm:** placebo in addition to usual care and medication once daily starting from trial entry and throughout pregnancy until day of delivery. |
| Comparator product(s) | Placebo |
| Maximum duration of treatment of a Subject | 1. The maximum duration will include the timespan from entering the trial to time of conception and length of pregnancy. The total maximum length of treatment is 21 months.<br><br>A maximum of 12 months treatment if a woman does not achieve pregnancy in this time period. |
| Version and date of protocol amendments | *Previous version:*<br>*Version 9.2, Date 31-JAN-2022*<br>*Version 9.3, Date 11-APRIL-2022*<br><br>*Current version: Version 10.0 12-DEC-2022* |

## 2. Glossary of Terms

| | |
|---|---|
| **Positive antiphospholipid (aPL) antibody test** | One of the following must be present on two occasions consecutively at least 12 weeks apart:<br>• Positive Lupus anticoagulant (LAC) by: aPTT, DRVVT, TSVT or Kaolin<br>• Anticardiolipin antibodies (aCL): IgG >95$^{th}$ percentile; IgM >95$^{th}$ percentile<br>• Anti-β2glycoproteinI (Anti-β2GPI): IgG >95$^{th}$ percentile; IgM >95$^{th}$ percentile<br>The last positive test must be within 12 months prior to enrollment and local reference ranges must be provided. |
| **Antiphospholipid syndrome (APS)** | The association of persistent positive antiphospholipid antibodies (at least 12 weeks apart) with venous and/or arterial thrombosis and/or microvascular thrombosis and/or pregnancy morbidity such as:<br>(a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology<br>documented by ultrasound or by direct examination of the fetus, or<br>(b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe preeclampsia<br>defined according to standard definitions, or (ii) recognized features of placental insufficiency, or<br>(c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded [1]. |
| **Refractory obstetric APS** | Women with persistent aPL who have experienced aPL related pregnancy complications despite treatment with aspirin and low molecular weight heparin. |
| **Placental insufficiency** | Generally accepted features of placental insufficiency include: (i) abnormal or non-reassuring fetal surveillance test(s), e.g. a non-reactive<br>non-stress test, suggestive of fetal hypoxemia, (ii) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, e.g. absent<br>end-diastolic flow in the umbilical artery, (iii) oligohydramnios, e.g. an amniotic fluid index of 5 cm or less, or (iv) a postnatal birth weight less than<br>the 10th percentile for the gestational age (see definition of fetal growth restriction) |
| **Fetal growth restriction (FGR):** | Fetal growth restriction as evidenced by an ultrasonographic estimated fetal weight based on<br>• Customized/population birth weight centile<br>• Birth weight <10$^{th}$ and <3$^{rd}$ customized/ population centile<br><br>According to Gardosi [2, 3]. |
| **Premature birth:** | Birth between 24 weeks + 0 days and 36 weeks + 6 days weeks gestation. |
| **Preeclampsia:** | Hypertension developing after 20 weeks gestation and the coexistence of one or more of the following new onset conditions:<br>1. Proteinuria (spot urine protein/creatinine >30 mg/mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/L ['2 + '] on dipstick testing)<br><br>2. Other maternal organ dysfunction:<br>• renal insufficiency (creatinine >90 umol/L)<br>• liver involvement (elevated transaminases and/or severe right upper quadrant or epigastric pain)<br>• neurological complications (examples include eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when<br>accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata)<br>• haematological complications (thrombocytopenia, DIC, haemolysis)<br>3. Uteroplacental dysfunction<br>• foetal growth restriction [4] |

<table>
<tr><td>Eclampsia:</td><td colspan="2">Onset of seizures in pregnant women with pre-eclampsia.</td></tr>
<tr><td>Gestational age</td><td colspan="2">Measure of the age of a pregnancy measured in weeks, from the first day of the woman's last menstrual cycle to the current date.</td></tr>
<tr><td>Apgar score</td><td>A routinely used measure of the physical condition of a newborn infant. It is obtained by adding points (2, 1, or 0) for heart rate, respiratory effort, muscle tone, response to stimulation, and skin coloration performed on a baby at 1 and 5 minutes after birth; a score of ten represents the best possible condition (The Apgar scoring system is shown to the right).</td><td>
<table>
<tr><th></th><th>0</th><th>1</th><th>2</th></tr>
<tr><td>HR</td><td>Absent</td><td><100</td><td>>100</td></tr>
<tr><td>Respiration</td><td>Absent</td><td>Irregular/ weak cry</td><td>Strong cry</td></tr>
<tr><td>Reflex irritability</td><td>No response</td><td>Grimace</td><td>Cough/ sneeze</td></tr>
<tr><td>Muscle tone</td><td>None</td><td>Some flexion</td><td>Well flexed</td></tr>
<tr><td>Color</td><td>Central cyanosis</td><td>Peripheral cyanosis</td><td>Pink</td></tr>
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</td></tr>
<tr><td>Demographic information</td><td colspan="2">Please record one of the following:<ul><li>British European, Pakistan, India, Poland, Bangladesh, Caribbean, Ireland, Chinese, Somalia, Iraq, Zimbabwe, Nigeria, Afghanistan, the Netherlands (Dutch European), Morocco, Turkey, Suriname, Antiles/Aruba, Germany, Ghana, Indonesia, Italy, Egypt, Romania, Albania, other North African, Peru, Philippines, Ecuador, Ukraine, Denmark, Sweden, Norway, Albania, Lebanon, Bosnia-Herzegovina, Other.</li></ul></td></tr>
</table>

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# 3. Background & Rationale

Antiphospholipid syndrome (APS) is the association of antiphospholipid antibodies (aPL) with both arterial and venous thromboses and/or obstetric morbidity (obstetric APS) [1].

We know that aPL are present in 20% of recurrent (three or more) first trimester miscarriages. Other obstetric complications are conditions associated with aPL-induced ischemic placental dysfunction, which include pre-eclampsia (PET), eclampsia, premature birth and fetal growth restriction (FGR) and stillbirth. Pregnancies in women with aPL are therefore considered as 'high risk', especially if women have a history of previous thromboses and/or obstetric complications. Current treatment regimens to prevent obstetric morbidity include low dose aspirin and low molecular weight heparin have improved pregnancy outcome but reduction in pregnancy loss remains sub-optimal.

Hydroxychloroquine is traditionally an antimalarial drug. Since the 1950's hydroxychloroquine has been widely used in the treatment of patients with rheumatoid arthritis or systemic lupus erythematosus (SLE). Hydroxychloroquine treatment of pregnant patients with SLE was first described more than three decades ago and its safety during pregnancy has been extensively documented and systematically reviewed [5-7]. APS is closely related to SLE, as up to 50% of patients with SLE have aPL[8].

Ostensen et al published their guideline for the use on anti-rheumatics in pregnancy more than a decade ago[7]. Their systematic review of several hundred pregnancies exposed to HCQ did not find any adverse pregnancy outcomes and recommended HCQ as the antimalarial of choice in women planning pregnancy and to be compatible with pregnancy and breastfeeding. In a recent update on this systematic review, which is the basis for the current British Society of Rheumatology (BSR) guidelines on HCQ in pregnancy, Flint et al. identified an additional 23 studies providing further information on 810 pregnancy exposures to HCQ. No specific pattern of congenital malformations was observed in association with HCQ exposure and it was concluded that HCQ is safe and is recommended as the 'immun-modulator of choice in pregnant women'[9]. These guidelines are in line with the European League against Rheumatism (EULAR) recommendations on the use of antirheumatics in pregnancy published in march 2017 [10].

The scientific basis for the use on HCQ is outlined below:

**What is known about HCQ's action in the presence of aPL: basic science**
Chloroquine and HCQ are weakly basic 4-aminoquinoline compounds. HCQ is the synthetic form of chloroquine and differs only by a hydroxyl group attached to a side chain resulting in a preserved efficacy with a less toxic side-effect profile [11]. The exact pharmacodynamics and action of HCQ *in vivo* remain to be fully uncovered, but most likely multiple molecular pathways are involved. HCQ's detailed effects in systemic lupus erythematosus (SLE) have been described elsewhere [12].

In retrospective clinical studies, HCQ was associated with a reduction in the risk of thrombosis in lupus patients and is discussed below. The mechanism by which HCQ might have an antithrombotic effect has been studied in a limited way. *In vitro* studies have showed that HCQ inhibits platelet aggregation and the release of arachidonic acid from aPL induced stimulated platelets [13]. Furthermore, Rand et al. showed

that aPL can disrupt the physiological anticoagulant shield of annexin A5 (AnxA5), leading to exposure of pro-coagulant phosphatidylserine and subsequently triggering thrombosis. As a novel finding, the group could show that HCQ restores the disruption of natural anticoagulant AnxA5 in patients with aPL [14].
Tissue factor (TF) is the key initiator of *in vivo* coagulation and has been implicated in in the pathogenesis of APS [15]. The ability of aPL to induce TF expression was demonstrated *in vitro* studies using serum samples from patients with purified aPL, which were added to cells, showing up-regulation of TF on monocytes [16, 17] neutrophils [18] and endothelial cells [19]. A number of investigators have found that serum, plasma, purified total IgG, antiB2GPI from APS patients increases TF expression and pro-coagulant activity on monocytes [20-22]. Lopez- Pedrera et al showed that anti-cardiolipin (aCL) IgG stimulate TF expression on circulating monocytes, acting through intracellular pathways including NFKappaB and MAP kinases [17]. In mice with aPL-induced fetal loss, neutrophils in turn can express TF through aPL–induced complement activation [23].
We have recently assessed the effect of using HCQ in patients with aPL and APS on plasma biomarkers including soluble TF, which has been shown to be increased in patients with APS. We showed that the use of HCQ was effective to decrease soluble TF levels in patients with aPL and APS 12 weeks after the commencement of HCQ compared to baseline [24]. This may be a mechanism contributing HCQ's antithrombotic effect.
Results from experiments in a *murine* model by Edwards et al. show that HCQ has the ability to reverse aPL-induced thrombosis [25]. The group observed that aPL-injected mice treated with HCQ had a significantly reduced thrombus size and that thrombus duration was reduced compared to mice treated with placebo [25]. *In vitro* studies showed that aPL are directly pathogenic towards trophoblast cells, which is a mechanism cited as a possible cause of recurrent first trimester pregnancy loss(es). *In vitro* studies have shown that HCQ reversed the aPL-induced inhibition of the chemokine interleukin 6 (IL-6) [26]. IL-6 released by first trimester trophoblast cells had been shown to drive trophoblast migration (a vital process for implantation) in previous studies by the same group [26].

Some studies support the hypothesis that the complement system is activated and plays a role in the pathogenesis of thrombotic and obstetric APS [23, 27-30]. Our group and others have previously confirmed complement activation in patients with isolated aPL and APS (thrombotic and obstetric APS) compared to healthy controls [30, 31]. Our most recent *in vivo* results assessing the effect of HCQ on complement activation markers C3-des-Arg and Bb in HCQ naive, non-pregnant aPL and APS patients at baseline and 12 weeks after the commencement of HCQ did not show any significant change in these values (Schreiber et al. The effect of treatment with hydroxychloroquine on biomarkers of hemostasis, complement, inflammation and angiogenesis in patients with antiphospholipid antibodies and antiphospholipid syndrome. Accepted in Rheumatology, 2017).
In mice exposed to complement inhibitors and knockout mice which have complement deficiencies there is inhibition of foetal loss and growth restriction mediated by aPL [23, 32] and also less aPL induced thrombus [27, 28]. Bertolaccini et al showed a possible protective effect HCQ of in a model of aPL-induced foetal loss [33]. In their mouse model, the fetuses died in 50% of pregnant aPL-injected mice and the survivors were growth-restricted and with smaller placentas compared to control mice. The administration of HCQ to mice exposed to aPL prevented fetal death, increased placental and fetal weight and decreased placental superoxide production (a marker of oxidative stress)[33]. In addition, the group reported HCQ-induced complement inhibition in APS patient serum, specifically looking at the

common pathway complement activation product C5a-des-Arg after receiving 6.5 mg/kg HCQ for 6 months [33].
At an intracellular level, *in vitro* studies show that aPL can induce endosomal NADPH (NOX) in endothelial cells and monocytes [34, 35]. NOX is an enzyme complex involved in pro-inflammatory signaling pathways [36]. Muller-Calleja et al recently published first *in vitro* data suggesting that HCQ significantly reduces the induction of endosomal NOX, which leads to a reduction of downstream gene activation [36]. This 'protective' mechanism of HCQ in preventing monocyte activation was confirmed in mice injected with human aPL [36].

**What is known about HCQ's use in patients with systemic lupus erythematosus and APS: clinical studies**
HCQ was the first drug licensed for the treatment of SLE and is one of the four currently licensed medications in SLE. The efficacy of HCQ in patients with SLE has been well described.
A cohort study of 150 SLE patients showed that it improves damage free survival [37], whereas data of 518 SLE patients from the observational LUMINA cohort showed that its use is associated with a reduced accrual of new disease damage. Ruiz-Irastorza performed a prospective observational cohort of 232 patients, showing an improved survival in SLE patients taking HCQ [38].
The antithrombotic effects of HCQ in patients with SLE was reported in data from the LUMINA cohort. In univariate analysis, including 442 SLE patients with aPL of whom 46 were identified having 51 recorded thrombotic events (over 1446 visits followed over a mean of 88 months), a protective effect of HCQ use was demonstrated (odds ratio [OR], 0.536)[39]. This finding is in line with an observational prospective cohort study of 232 SLE patients, in which Cox regression analysis showed that HCQ use was associated with a reduced risk thrombosis (seven events occurred while patients were taking HCQ, 7 further events happened after the patient had stopped HCQ whereas 28 events were reported in patients who had never taken HCQ, so yielding a hazard ratio [HR] of 0.28) [38]. Likewise, the antithrombotic effect of HCQ could also be demonstrated in several other cohort, case-control and retrospective studies [12].

The role of HCQ in APS is currently being investigated in thrombotic and obstetric APS. In a prospective non-randomized study of 40 patients with primary thrombotic APS without underlying SLE, Schmidt-Tanguy et al. studied the effect of 400 mg HCQ in addition to oral anticoagulation with vitamin K antagonists (VKA), target INR 2-3. None of the patients receiving HCQ along with standard anticoagulation had recurrent thromboembolic events, whereas 30% in the control group experienced a recurrent event (p=0.0086)[40].
In retrospective studies of pregnant patients with aPL and or APS, we and others showed that HCQ is a candidate for preventing aPL-related adverse pregnancy outcomes [41, 42].
In a retrospective multicenter cohort consisting of 30 APS patients (and 35 pregnancies), Mekinian et al reported that HCQ was associated with fewer first trimester miscarriages (pregnancy losses decreased from 81% to 19%, $p<0.05$) and improved live birth rates in refractory obstetric APS to 78% ($p<0.05$)[41]. In our retrospective observational cohort study of 96 women with 170 pregnancies, we also showed that HCQ treatment was associated with a higher rate of live births (67% in HCQ treated vs. 57% in untreated patients, p=0.05) and a lower prevalence of pregnancy morbidity (47% vs. 63%, p=0.004). Pregnancy duration was longer in patients receiving HCQ compared to those who did not receive HCQ (median 27.6 weeks, range [6-40] versus 21.5 weeks [6-40], p=0.03) and foetal losses beyond the 10 weeks of gestation were less frequent in women who were treated with HCQ (2%

vs. 11%, p=0.05). Moreover, ischaemic placental mediated complications (pre-eclampsia, eclampsia and foetal growth restriction (FGR) were less prevalent in HCQ treated women than in the control group (2% vs 10.9%, p=0.05). There was a significantly higher rate of women undergoing spontaneous vaginal labour in HCQ women compared to women without HCQ treatment (37.3% vs. 14.3%, p=0.01). The association of HCQ with the absence of aPL-related complications in pregnancy was confirmed in multivariate analysis (OR 2.2; 95% CI 1.2-136.1; p=0.04)[42]. In none of the above mentioned studies any fetal abnormalities were observed [41, 42].
We also conducted a systematic review of the evidence of HCQ in obstetric APS, which confirmed a lack of evidence. We therefore performed an expert based clinical judgement consensus, an accepted approach to address a specific and clinically relevant question in an area were best clinical practice is uncertain. The experts agreed that HCQ could be of benefit in certain circumstances in pregnant women with aPL [43].

The only solution to assess the effect of HCQ on pregnancy outcomes in women with aPL is to conduct a randomized controlled trial (RCT) of HCQ versus placebo. This trial will be the first of its kind and will lead to evidence-based recommendation on the use of HCQ in women with aPL.

# 4 Trial Objectives and Design

## 4.1. Trial Objectives

1. To study the effect of hydroxychloroquine on pregnancy outcome in women antiphospholipid antibodies.

### To collect blood samples for pharmacokinetic and biomarkers studies

### 4.1.1 Primary endpoints

The primary endpoint is a composite of three principal aPL-related adverse pregnancy outcomes: one or more miscarriage (either < 10 weeks gestation or between 10 and 27+6 weeks gestation) and premature birth before 34 weeks due to any of: pre-eclampsia, eclampsia, fetal growth restriction (FGR) or stillbirth (i.e. after 24 weeks).
Premature birth for other reasons will not be included.

The components of the primary endpoint will each be presented as secondary endpoints (below).

### 4.1.2 Secondary endpoints

The pre-defined secondary endpoints include:

The pre-defined secondary endpoints include:
1. Pregnancy loss < 10 weeks gestation
2. Pregnancy loss > 10$^{th}$ week of gestation of a morphologically normal fetus documented by ultrasound or by direct examination of the fetus
3. Premature birth of a morphologically normal neonate < 34 weeks due to any of: pre-eclampsia, eclampsia, recognized features of placental insufficiency.
4. Gestational age at delivery
5. Birth weight
6. Delivery by Caesarean section
7. Apgar score < 7 at 5 min
8. Neonatal morbidity (bleeding or thrombotic complications, infections, congenital abnormalities)
9. Days to hospital discharge following delivery (mother & child)
10. Thrombotic events in the mother during pregnancy and 6 weeks post-partum.
11. Days of neonate in special care
12. Safety and tolerability of hydroxychloroquine in the mother and in the neonate

## 4.2 Trial Design

This is a double blind randomized controlled trial (RCT) of women with aPL planning to fall pregnant, who will be randomized to hydroxychloroquine or an identically looking placebo in addition to their usual medication.
According to our sample size we will enroll 328 pregnant women. In order to achieve this number of patients we will randomize 400 patients.
Our hypothesis is that hydroxychloroquine improves aPL-related pregnancy complications.

The HYPATIA trial has been designed as a multicenter RCT. Participating centers in the UK include Guy's and St Thomas' NHS Foundation Trust, University College London NHS Foundation Trust; the Royal Free London Hospitals, Liverpool Women's Hospital NHS Foundation Trust; Manchester University Hospital, Oxford University Hospital and Cambridge University Hospitals.
In the EU participating centres include: Copenhagen, Hvidovre, Odense, Århus, Ålborg, Sønderborg (all in Denmark), University Hospital Torino/Italy; and Dublin University Hospital/Ireland, Calgary University Hospital/Canada. The HYPATIA trial is supported by an accredited Clinical Trials Unit (King's College London Clinical Trials Unit).

Each center runs a dedicated pregnancy clinic following a routine care protocol for pregnant women with aPL. The patients are closely monitored during their pregnancy and are followed up at least once every trimester, if needed more closely. A visit assessment schedule is outlined in on page 16. The study procedures for the HYPATIA trial are outlined specifically and are almost identical with the standard of care for these patients. We expect all participating sites to follow these study procedures. Data from the routine clinical visits prior to study entry may be used in the HYPATIA study. This includes data from standard of care samples, such as blood results (full blood count, biochemistry, liver profile, auto-antibody status (i.e. Lupus anticoagulant, anti-cardiolipin antibodies, anti-β2-glycoprotein-I, ENA antibodies).

For women entering this trial additional hospital visits are therefore not required, as trial visits will coincide with the standard of care visits. Women who decide to participate in the HYPATIA trial will have their usual medication, with hydroxychloroquine or placebo as additional therapy. Women will have a routine blood test assessing full blood count, kidney and liver function and a physical exam (including heart and lung stethoscopy) every 3 months before becoming pregnant and once every trimester. This blood test will coincide with their usual follow-up/study visit and women do therefore not need to attend more often.

*Only for women in selected centres:* Women attending selected centres will be invited:

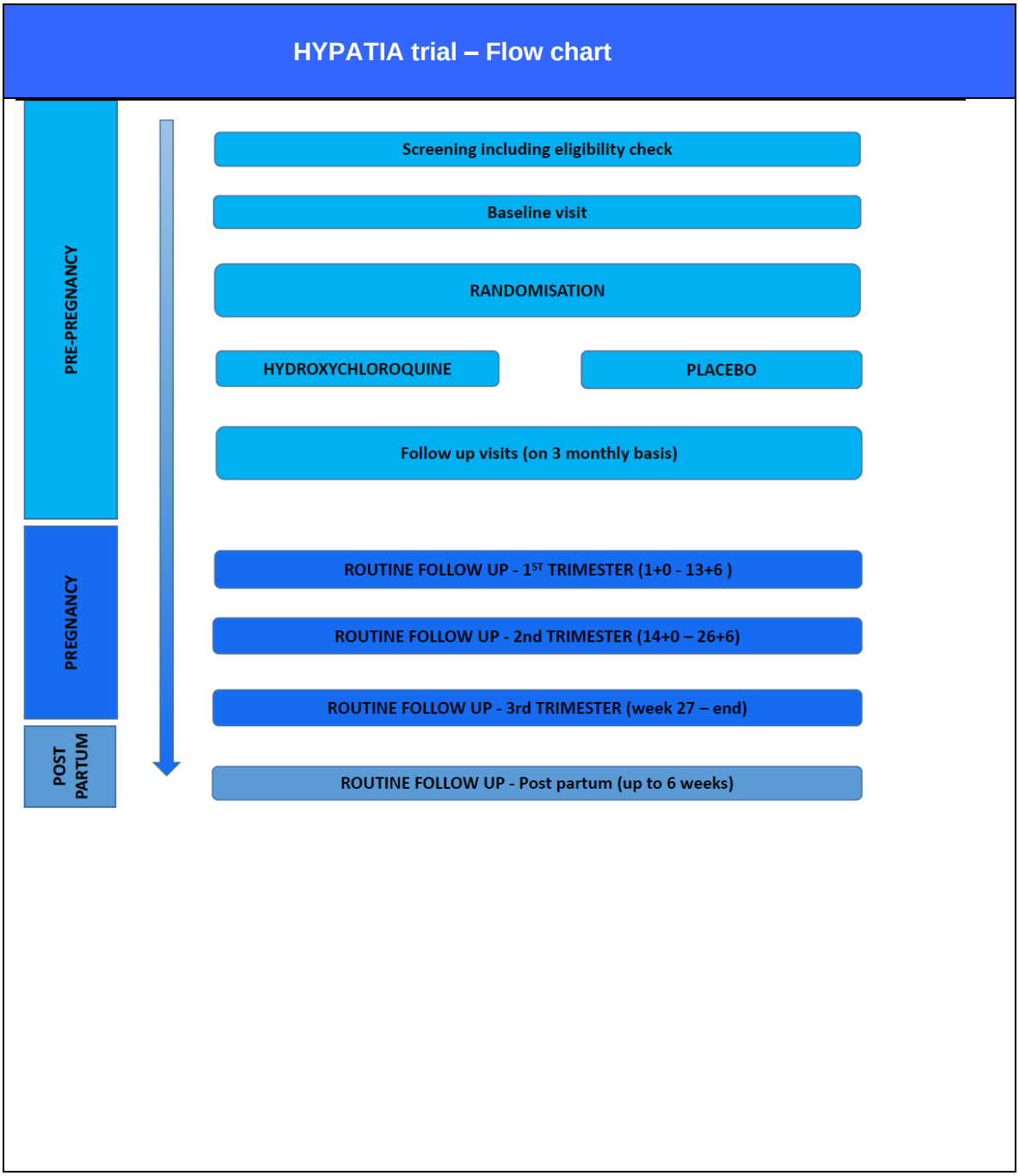
- To donate blood samples in order to assess 1. biomarkers and 2. the pharmacokinetics of hydroxychloroquine in pregnancy.

These data will be analyzed separately from separate funding sources and do not form part of HYPATIA trial.
An overview of the selected centres is given below:

| Centre | Country | HYPATIA study only | Option for research bloods |
|---|---|---|---|
| Rigshospitalet | Denmark | x | x |
| Hvidovre | Denmark | x* | |
| Odense | Denmark | x | x |
| Århus | Denmark | x | x |
| Ålborg | Denmark | x | x |
| Sønderborg | Denmark | x* | |
| GSTT, London | UK | x | x |
| Royal Free, London | UK | x | |
| UCLH, London | UK | x | |
| Royal Free, London | UK | x | |
| Oxford | UK | x | |
| Cambridge | UK | x | |
| Liverpool | UK | x | |
| Manchester | UK | x | |
| Turin | Italy | x | x |
| Dublin | Ireland | x | x |
| Calgary | Canada | x | x |
| *sites only screen for eligibilty (but no randomisation) | | | |

The trial will be carried out in accordance with the protocol and current statutory requirements/legislation.

## 4.3 Trial Flowchart




**HYPATIA trial study procedures**

| | Pre-pregnancy | | | Pregnancy | | | Post-partum |
|---|---|---|---|---|---|---|---|
| | Screening visit* | Baseline visit* | Pre-pregnancy visits § | 1st Trimester | 2nd Trimester | 3rd Trimester | Post- partum visit† |
| **Written informed consent** | x | - | | | | | |
| **Demographics** | x | | | | | | |
| **Eligibilty assessment (1)** | x | x | | | | | |
| **Past medical history (2)** | x | | | | | | |
| **Smoking history** | x | | | | x | | |
| **Past pregnancy history (3)** | x | | | | | | |
| **Current medication** | x | x | x | x | x | x | x |
| **Height** | x | | | | | | |
| **Weight** | x | | | | | | |
| **Collection of blood results (4)** | x | | | | | | |
| **Blood pressure & Pulse** | x | | | | | | |
| **Urine dip** | x | | | | | | |
| **Randomisation** | | x | | | | | |
| **Dispensation IMP** | | x | x | x | x | x | |
| **Adverse events monitoring** | | x | x | x | x | x | x |
| **Physical exam (incl stetoscopy)** | x | | x | x | x | x | |
| **Blood sample (5)** | x | | x | x | x | x | |
| **Info about current pregnancy (6)** | | | | x | x | x | |
| **Compliance check (7)** | | | x | x | x | x | x |
| ***Blood for additional research (8)*** | | *x* | *x* | *x* | *x* | *x* | *x* |
| **Growth scans** | | | | | x | x | x |
| **Uterine Artery Doppler (20-24 weeks)** | | | | | x (9) | | |
| **Child gender** | | | | | | | x |
| **Birth date** | | | | | | | x |
| **Child APGAR at 5 min** | | | | | | | x |
| **Days of admission (mother&child)** | | | | | | | x |
| **Gestational age** | | | | | | | x |
| **Mode of delivery (Cesarean Y/N)** | | | | | | | x |
| **Birth weight** | | | | | | | x |
| **Neonatal morbidity (neonatal bleeding or thrombotic complications, infections, congenital abnormalities, days in neonatal special care)** | | | | | | | x |

* These visits can all be completed on the same day. Consent within 3 months.

§ Visits every 3 months until patient pregnant, then new dispensation of study drug and dispensation every 3 months. Pre-pregnancy visits max 4 times (a total of 12 months)

† Follow up visit should be completed within 6 weeks. If the patient is unable to attend clinic within the timeframe of 6 weeks, the study team can contact the participant to obtain the study information.

(1) Eligibility assessment: persistent presence of aPL twice 12 weeks apart, ENA negative

(2) Past medical history as per exclusion criteria & detailed history about previous venous thrombosis, previous arterial thrombosis and any other significant past medical history. Blood sample review (FBC, kindney and liver profile) and results will be collected: aPL antibodies, ENA antibodies. Blood results can be obtained from routine blood results prior to entering the HYPATIA study. Otherwise they will be done at screening as per standard of care.

(3) Past pregnancy history - to record in chronological order : Year, birthweight, gestation, miscarriage < 10 weeks, miscarriage > 10 weeks, premature birth < 34 due to pre-eclampsia, eclampsia, fetal growth restriction or intrauterine death (>24 weeks).

(4) max 12 months old (antiphospholipid antibodies (aPL) type and titres incl local cut off, ENA antibodies). Repeat aPL if needed.

(5) blood sample include full blood count, kidney and liver function as standard of care. For the screening bloods, in case any results are available and are less than 3 months old they can be used.

(6) IVF pregnancy (Y/N), singleton/twin or triplet pregnancy, current gestation, estimated date of delivery

(7) Compliance check (Kit number to be noted, date of current kit started & ended, number of tablets taken, number of tablets missed)

(8) Only for patients in selected centres: optional research bloods

(9) Data can be collected on 2nd or 3rd Trimester visit. All scans should be recorded on eCRF.

# 5 Trial Medication

## 5.1 Investigational Medicinal Product (IMP)

1. Active **hydroxychloroquine description**:

The active is hydroxychloroquine 200mg film coated tablets. The tablet is presented a white, circular, biconvex film coated tablet debossed with '200' on one side and plain on the other side (Quinoric®, MA holder: Bristol Laboratories Ltd (tablets PL 17907/0017). A list of excipients can be found in the Summary of Product Characteristics (SmPC).

The active product is deblistered from blister packs, packed, labelled, and UK QP released and distributed to Great Britain (GB) study sites by Guy's and St Thomas' NHS Foundation Trust, Pharmacy Manufacturing Unit (MIA IMP: 11387).

2. Placebo **description**: The placebo is a white, circular, biconvex tablet debossed with '200' on one side and plain on the other side to match theappearance of Hydroxychloroquine 200mg film-coated tablets, PL 17907/0017. The Simplified Investigational Medicinal Product Dossier (sIMPD) details the composition of the placebo.

The placebo product is manufactured, packed, labelled, and UK QP released and distributed to Great Britain (GB) study sites by Guy's and St Thomas' NHS Foundation Trust, Pharmacy Manufacturing Unit (MIA IMP: 11387).

The Sponsor has contracted with Sharp Clinical Services to provide EU import and EU QP release, as well as distribution of the IMP to EU study sites. These activities will be performed by Manufacturing Packaging Farmaca (MPF) B.V., Heerenveen, The Netherlands (Manufacturers Authorisation: 108630F) on behalf of Sharp Clinical. An additional agreement for IMP shipment will be made for Calgary in Canada.

The Kings Clinical Trials Unit (KCTU) Intervention Management System will be used to allocate blinded supplies to patients. The study team, site pharmacy team and patient will remain blind to the treatment allocation.

The KCTU trials pharmacist will be unblinded and will have access to the KCTU Intervention Management System to maintain IMP stock levels, both centrally and at study sites. This individual will be responsible for placing orders for delivery to study sites.

**Packaging and labelling:**

The active and placebo tablets will be packed into Annex13-labelled, high-density polyethylene (HDPE), tamper-evident, child-resistant closure bottles. Each treatment pack will contain 96 tablets and will have a unique treatment pack number.

Multi-language labels will be applied to the bottles, allowing the IMP to be shipped to any recruiting site globally. The Sponsor will be responsible for approving the label content in each country.

Once randomized, a unique pack number in the trial arm that the participant has been randomized to, will be automatically allocatedin the KCTU Randomization and Intervention Management system. A copy of the randomisation notification will be emailed to all individuals that have been given access to receive these details. A study specific prescription will be sent to the dispensing pharmacy, along with a printed copy of the randomization email. At each subsequent visit, study site staff will access the KCTU Randomization and Intervention Management system and allocate a new unique pack number for the participant. Study site pharmacists must cross check the prescription and the randomization notificationat each dispensing episode to ensure the correct pack number is dispensed. Study medication will be dispensed at randomization and quarterly thereafter until month 9 or until pregnancy is confirmed. Once pregnancy is confirmed, a new pack is dispensed. During pregnancy study medication will continue to be dispensed on 3-monthly basis until pregnancy end.

In exceptional circumstances (such as lockdown during Covid19 pandemic), the dispensedIMP can be sentto the participant by post and the participant will not have the physical exam or blood tests at that time but will have the routine clinical exam and blood test at the subsequent study visit.

## 5.2 Dosing Regimen

Patients will be randomised to either hydroxychloroquine 200 mg once daily or an identical placebo, which is to be taken in addition to the participants usual medication. Hydroxychloroquine should ideally be taken with a meal or a glass of milk.

The duration will include the time span from entering the trial to time of conception and length of pregnancy.

The maximum pre-pregnancy treatment period is 12 months in case a woman does not achieve pregnancy in this time.

Therefore, the maximum duration of treatment for a participant can be 21 months (7 dispensing visits) including the length of pregnancy. Refer to list of study visits below:

- Pre-pregnancy Randomisation (baseline visit)
- Pre-pregnancy Month 3
- Pre-pregnancy Month 6
- Pre-pregnancy Month 9
- Pregnancy Trimester 1
- Pregnancy Trimester 2
- Pregnancy Trimester 3

## 5.3 IMP Risks

Hydroxychloroquine is traditionally an antimalarial drug and since the 1950's has been widely used in the treatment of patients with rheumatoid arthritis or systemic lupus erythematosus (SLE). Hydroxychloroquine treatment of pregnant patients with SLE was first described more than three decades ago and its safety during pregnancy has been extensively documented and systematically reviewed [5-7].

The European Medicines Agency has licensed hydroxychloroquine as orphan medical product for the treatment of APS (EU/3/16/1820). We are aware that hydroxychloroquine is labelled as 'contraindicated in pregnancy' according to the SmPC. However, it is widely used in women during pregnancy and lactation and is recommended for the use in pregnancy by European and British Societies (the European League against Rheumatism (EULAR) and the British Society for Rheumatology (BSR)).

Dr Schreiber is a member of the BSR working group on 'prescribing antirheumatic drugs in pregnancy'. Their guidance has been published in *Rheumatology* [9, 44]. After reviewing more than 810 pregnancies in the literature where women were prescribed hydroxychloroquine through pregnancy, they recommended that hydroxychloroquine is the antimalarial drug of choice in pregnant patients with autoimmune diseases and they did not identify significant risks for intrauterine exposure to the fetus. According to these NICE approved BSR guidelines, hydroxychloroquine is the antimalarial of choice for pregnant patients requiring immune modulation [9, 44]. The BSR guideline has been updated in November 2022, as hydroxychloroquine is still the antimalarial of choice for pregnant women and deemed safe before conception, during pregnancy and also during breastfeeding [45, 46]. Safety assessments for mother or baby are not recommended.
Further supporting the safety of hydroxychloroquine in pregnancy and lactation, the EULAR have very recently published their European recommendations on the use of hydroxychloroquine in pregnancy and lactation. The EULAR group highlighted that hydroxychloroquine is safe in pregnancy and that there is no evidence of an increased risk of malformations in children exposed to hydroxychloroquine, that hydroxychloroquine can be continued throughout pregnancy and during breastfeeding [47].

Use of hydroxychloroquine in patients with psoriasis and porphyria may precipitate a severe attack. These patients are therefore excluded from the HYPATIA study. Retinal toxicity has been described with high dose hydroxychloroquine, however, low doses of hydroxychloroquine (less than 6.5mg/kg bodyweight) are very rarely associated with retinopathies. Patients participating in the HYPATIA trial will be receiving hydroxychloroquine 200 mg once daily, which is half of the maintenance dose according to the SmPC, or an identical placebo. For a patient weighing 75 kg, a daily dose of 200 mg is equivalent 2.6 mg/kg body weight and this dose has been shown not to be associated with retinal toxicity [48-50]. Women with a body weight less than 45 kg are not eligible for the trial

**What is known about the dosing of HCQ in pregnancy:**
According to the SmPC a dose of 200 mg hydroxychloroquine is the low maintenance dose for the indication of rheumatoid arthritis, discoid and systemic lupus erythematosus.
Several hundred pregnancies exposed to hydroxychloroquine 200 to 400 mg daily during the first trimester did not find an increase in congenital malformations including cardiac conduction disturbances in children exposed antenatally to hydroxychloroquine as reported by Ostensen et al in 2006 [9, 51]. The BSR working

group on the use of antirheumatics in pregnancy, of which Dr Schreiber is a member, identified an additional 23 studies; 2 systematic reviews [52, 53], 2 case controls [54, 55], 10 cohort [56-65], 3 case series [66-68] and 6 case reports [69-74] providing further information on 810 pregnancy exposures to 200 – 400 mg HCQ. The BSR working group has in November 2022 updated their guideline, and still recommends that HCQ is safe in pregnancy.
In two more recent retrospective clinical studies of HCQ in patients with obstetric APS, patients were also exposed to a dose of 200mg – 400mg hydroxychloroquine daily and no adverse events in the neonates were reported [41, 42].

When reviewing the literature on the use of 200 mg daily, several studies have focused on possible long-term effects of HCQ in children exposed in utero or during lactation (in one study the average daily maternal dose of hydroxychloroquine was 317 mg and in the second study and in the second study the daily dose was 200 mg) [75, 76]. No decrease in visual acuity, visual field or colour vision, changes in electroretinogram and electro-oculogram were detected in a series of six children studied during the first year of life and up to 4 years of age [77]. A case-control study including 133 pregnancies exposed to hydroxychloroquine found no visual, hearing, growth or developmental abnormalities in children followed up for 108 months. Electrocardiograms of exposed children were normal [51, 78].

**What is known about the pharmacokinetics of hydroxychloroquine in pregnancy:**
Hydroxychloroquine is a diastereoisomer, has basic properties and is given as the sulphate. While being relatively well absorbed orally and with good bioavailability, hydroxychloroquine has a long and variable plasma terminal elimination half-life (approximately 40-60 days). Hydroxychloroquine undergoes renal clearance [79]. HCQ passes across the placenta, with cord blood concentrations nearly identical to those found in maternal blood, suggesting that during pregnancy the level of exposure to HCQ in mother and fetus is similar[80]. A systematic review including a meta-analysis of hydroxychloroquine exposed pregnant patients with autoimmune diseases including five studies did not show any increased risk of congenital malformations in babies born to mothers who were exposed to hydroxychloroquine in pregnancy[53]. These findings are in line with the more recent systematic review of anti-rheumatics in pregnancies published by Flint et al[9, 44]. To the best of our knowledge no studies exist to inform on the plasma levels of patients exposed to hydroxychloroquine during pregnancy.

**Length of hydroxychloroquine exposure:**
The maximum length of hydroxychloroquine exposure for women participating in the HYPATIA study is 12 months + the individual pregnancy length, i.e. a maximum total length of 21 months hydroxychloroquine exposure.

The main concern associated with hydroxychloroquine exposure is the development of retinopathy[81]. A number of retrospective and prospective studies have attempted to assess the risk of developing HCQ retinopathy and to identify risk factors for toxicity[48, 82]:
In the largest study to date, Wolfe and Marmor screened 3995 patients who had received HCQ for SLE or RA for self-reported toxicity, followed by specialist confirmation of these cases. In this study definite or probable HCQ toxicity was noted in 0.65% (95% CI: 0.31, 0.93%). They reported a clear increased risk with duration of exposure: <0.3% for those with HCQ exposure of less than 5 years but up to 2% for those with exposure of 10 to 15 years. Interestingly, they found no association with

daily dosage, but they note that their data regarding this was incomplete[82]. Levy's retrospective chart review of 1207 patients receiving HCQ found no cases of definite toxicity in patients on HCQ <6.5 mg/kg/day, but one patient with definite toxicity and five with indeterminate but probable toxicity in those who received >6.5 mg/kg/day HCQ[48]; the patient with definite toxicity had taken HCQ for 7.3 years at a dose of 6.98 mg/kg/day. Overall probable toxicity occurred in 0.5% of patients in the Levy series, a similar proportion to the Wolfe study.
Smaller studies include those of Mackenzie[83], who found no cases of HCQ retinopathy in a cohort of >900 patients with rheumatoid arthritis on HCQ and Wang's study[84], which reported one patient with HCQ retinopathy in 156 patients with SLE who had taken HCQ, and that patient had previously taken HCQ for 6 years at a dose 6.5 mg/kg/day[84].
In a more recent retrospective study on 2361 patients, the prevalence of hydroxychloroquine related retinopathy was estimated higher at 7.5% and depending on dose and duration of therapy can increase to 20-50 % after 20 years of therapy using more sensitive testing methods[85].

Prospective studies however support a low rate of HCQ toxicity. Mavrikakis and colleagues reported on their prospective series of 526 patients who received HCQ, of which 400 patients had received at least 6 years of treatment. Prior to 6 years of treatment, no patients had developed retinopathy. Two patients subsequently developed retinopathy (one at 6.5 years and one at 8 years), equating to a rate of 0.5% for those patients with over 6 years of treatment. The mean duration of treatment in this group was 8.7 years, indicating a relative paucity of patients with longer follow-up[86].
Accurately estimating the incidence of HCQ retinopathy is challenging. Most studies are case series or retrospective cohorts, and the relatively few prospective studies are limited in size and duration of follow-up. The data available does, however, suggest that HCQ retinopathy remains rare especially if patients are treated with doses less than 5 mg/kg and less than 5 years. In our HYPATIA study women will be maximally exposed for 21 months in total and as we are excluding women weighing less than 45 kg (200 mg once daily in a patient weighing 45 kg equals 4,4 mg/kg).
Other data on adverse effects in relation to exposure length are limited. To the best of our knowledge the only study assessing exposure length in relation to toxicity towards the myocardium is published by Costedoat-Chalumeau et al, who in a series of 85 patients with connective tissue disease evaluated heart conduction disorders with electrocardiograms (ECG). Patients were exposed between 12 months up to 50 months, and the group could not show any significant difference between cases and controls[87].

The Royal College of Ophthalmologists (RCOphth) guideline published in 2018 recommended to conduct a baseline eye exam (incl. visual fields test and optical coherence tomography) in patients who start HCQ therapy and in whom it was planned to continue treatment for at least 5 years. However, these 2018 guidelines have been updated in December 2020 (REF), and **baseline retinal monitoring is no longer** recommended. The advice on baseline monitoring has been reviewed on the basis of a large UK audit of 782 individuals who had taken hydroxychloroquine for less than five years. It found that none had an abnormality on testing that would have meant stopping treatment. The guidelines also point out that a significant proportion of those who start taking hydroxychloroquine are no longer prescribed it five years later.

Patients with a known or newly diagnosed retinopathy will not be included into the study, and women will be therefore asked at study entry if they have any new visual problems. Those with visual problems will be referred to a formal eye exam.We also

exclude patients with renal insufficiency (renal replacement therapy) as this may lead to in increased plasma levels of hydroxychloroquine. We are also aware the SmPC states that hydroxychloroquine has been associated with cardiomyopathy, and that caution has to be taken in patients with severe neurological or blood disorders. Patients in the HYPATIA trial will be monitored with physical examinations and blood tests to ensure their safety. All trial teams are aware of the possible side-effects and in case patients describe symptoms that may be related to hydroxychloroquine side-effects these will be investigated accordingly.
As hydroxychloroquine may precipitate a porphyria attacks and worsen psoriasis, these patients will therefore be excluded from the HYPATIA study. We will advise patients, that hydroxychloroquine may alter the need for glycaemic control in those with diabetes.

The table indicates hydroxychloroquine's potential side-effects according to the SmPC. We will ensure all participants safety and use the following modalities to screen and monitor any potential side-effects:

| **Side-effects according to SmPC** | **Frequency according to SmPC** | **Modality to monitor/screen** |
|---|---|---|
| Retinopathy | Uncommon | - Pt with retinopathy will not be included as per exclusion criteria, and women will therefore be asked if they experience any visual problems prior to study entry (if this is the case they will be sent to a formal eye test)<br>- |
| Cardiac effects (cardiac failure) | Uncommon | - Regular heart and lung stethoscopy |
| Hepatic or renal illness | Unknown | - Pts with known hepatitis or renal failure will not be entered into the study<br>- Liver and renal function will be monitored every three months<br>- Pts in whom renal function deteriorates (i.e. creatinine clearance of less than 30 ml/min) will be withdrawn from the trial |
| Severe gastrointestinal, neurological or blood disorders | Unknown | - Pts with severe gastrointestinal, neurological or blood disorders will not be entered into the study |
| Glucose-6-phosphate dehydrogenase (G6PD) deficiency | Unknown | - Pts with G6PD deficiency will not be entered into the study and those of Mediterranean origin or a family history will be screened as per standard of care |
| Bone marrow depression | Rare | - Regular full blood count (includes red and white blood cells and thrombocytes) |
| Hypoglycaemia | Unknown | - Pt with diabetes on antidiabetics will be made aware of this side-effect and we will ask women to communicate this to their diabetes care givers |
| Musculoskeletal effects (patients on | Unknown | - Physical exam of muscle strength (shoulder and hip girdle) |

| | | |
|---|---|---|
| long-term treatment according to SmPC) | | |
| Dermatological reactions (Steven Johnson syndrome and toxic epidermal necrolysis) | Unknown | - Pt will be made aware of these side-effects (mentioned in ”Patient info sheet’) and will be asked if they have experienced any skin rashes. Pts will be informed to stop treatment in case of progressive skin rash with blisters) |
| Hereditary problems of galactose intolerance, the lactase deficiency or glucose-galactose malabsorption | Unknown | - Pts with galactose intolerance, the lactase deficiency or glucose-galactose malabsorption will not be entered into the study |
| Ototoxicity in the newborn | Limited data | - All babies will undergo the usual exam as part of the NHS routine care. No extra exam is recommended. |
| Retinal toxicity in the newborn | Limited data | - No extra exam is recommended. |

**Newborn screen for potential retinal and oto-toxicity:**
In babies exposed to potentially retino or ototoxic drugs, we were initially asked to perform a direct ophthalmoscopy and a diagnostic Auditory Brainstem Response (ABR) test at 4kHz – a high frequency sound along with diagnostic transient-Evoked otoacoustic emissions (TEOAE) which are objective tests more sensitive than the newborn hearing screen.
However, all major updated guidelines from societies relevant to the field have been updated since our first protocol proposal in 2017. The societies including the British Society for Rheumatology from 2022 (BSR), the British Society for Ophthalmology 2021, the American College of Rheumatology 2020 (ACR) and The European Alliance of Associations for Rheumatology formerly the European League Against Rheumatism 2017 (EULAR) recommend no baby eye or ear screening [10, 45, 46, 88, 89].
As a direct ophthalmoscopy and ARB and a TEOAE are exams that take time, and requires the babies to comply with the exam technique (the baby needs to sleep for the hearing test), and none of the babies born of mothers participating in the study as of November 2022 have had abnormal hearing eye tests, we therefore exclude the eye and ear screen of the babies born to mothers participating in the HYPATIA study from the current protocol version 10.0.

## 5.4 Drug Accountability

The pharmacy clinical trials team must maintain accurate accountability records including, but not limited to, the number of bottles/tablets received, the number of bottles/tablets and bottle numbers (i.e., unique treatment pack number) dispensed to each participant, batch number, expiry date, and the date of the transaction in addition to the quantity of investigational product returned by each patient. A Sponsor accountability log (includes both inventory/subject level details) will be provided to study sites.

Participants will be asked to return any unused IMP and/or empty packaging at each study visit. The study drug returns will be returned to pharmacy by the research team

for accountability. The returns will be verified by the pharmacy clinical trials team and the Clinical Research Associate (CRA)/dedicated research member prior to disposal at site. Destruction of IMP must be in accordance with the site IMP destruction Standard Operating Procedure (SOP). Disposal of IMP is only permitted with sponsor authorisation.

The pharmacy clinical trials team are required to maintain copies of study drug shipping receipts and drug accountability records in accordance with regulatory requirements.

## 5.5 Storage of IMP

The IMP must be stored at room temperature, between 15°C to 25°C, in a secure area with limited access. Protect from moisture.

Appropriate storage conditions must be ensured by completion of temperature logs in accordance with local requirements. As a minimum requirement, min/max thermometers readings must be recorded each day of the working week, Mondays to Fridays. The temperature logs can be stored centrally, and records made available for review to the sponsor CRA as requested.

If the IMP is exposed to temperatures outside of 15°C to 25°C, all supplies must be quarantined and the CRA emailed immediately, copying in the Trial Manger. Await further instruction from the Sponsor.

## 5.6 Subject Compliance

All participating patients will be instructed about the importance of medication compliance. Compliance with treatment will be monitored at every follow up visit and patients will be asked to bring back their empty medication packaging. Remaining tablets will be counted by the treating physician and will be recorded in the patient CRF.

Patients should be informed to contact the trial teams in case of accidental medication loss.

All patients will be asked not to modify the treatment without discussion. If a patient intends to interrupt treatment with IMP, they will be asked to contact the trial team prior to doing so. If a patient decides to stop the treatment without speaking to any trial members, this will be recorded at the next follow up appointment. The patient will remain in the study unless they chose to formally withdraw from the study.

## 5.7 Concomitant Medication

We are proposing a study of hydroxychloroquine in addition to their usual medication in women with underlying aPL who are planning pregnancy. As with chloroquine, antacids may reduce absorption of hydroxychloroquine, so we advise a four hour interval between the IMP and the intake of any antacids.

As hydroxychloroquine may enhance the effects of a hypoglycaemic treatment, a decrease in doses of insulin or antidiabetic drugs may be required.

All patients will very likely be on some form of medications prior to entering this trial, which may include aspirin, heparin, in some particular cases warfarin or immunosuppressant medications such as azathioprine. (Warfarin may be used as secondary thrombosis prevention, however, once they have a positive pregnancy test, the warfarin will be stopped and they switched to low molecular heparin according to usual medical practice). An individual assessment will be made prior to entering the trial. None of those drugs are contraindicated as concomitant therapy with hydroxychloroquine. All drugs patients are taking will be recorded at every visit in the patient CRF. This information includes drug type, strength, how long the medication was taken for and the indication.

Patients participating in this trial will either be planning for or be pregnant. All medications absolutely contraindicated in pregnancy should be avoided after pregnancy is confirmed. In this particular patient group this may for example include anticoagulation treatment (such as warfarin) or certain types of blood pressure medications.

# 6 Selection and Withdrawal of Subjects

## 6.1 Inclusion Criteria

1. Women with known aPL (ie. isolated aPL or APS) who are planning pregnancy. aPL are defined by the presence of a positive test for anticardiolipin antibodies (IgG/IgM isotypes > 95$^{th}$ percentile) and/or lupus anticoagulant and/or anti- beta 2 glycoprotein-I (IgG/IgM isotypes > 95$^{th}$ percentile), on two or more consecutive occasions more than 12 weeks apart (a positive aPL test is defined under ‘glossary and definitions’). The last positive test must be within 12 months of study entry.
2. Written informed consent to participate

## 6.2 Exclusion Criteria

1. Women who are already pregnant
2. Allergy or adverse event to hydroxychloroquine. Hypersensitivity to the active substance, 4-aminoquinoline or any of the compounds of the IMP or placebo.
3. Current treatment with hydroxychloroquine
4. Age < 18 or > 45
5. Body weight < 45 kg
6. Psoriasis
7. Uncontrolled epilepsy
8. Anti-Ro antibodies
9. Renal replacement therapy
10. Other severe active co-morbidities (HIV, hepatitis B, severe gastrointestinal, neurological or blood disorders)
11. Porphyria

12. History of retinopathy or newly diagnosed retinopathy
13. History of galactose intolerance, lactase deficiency or glucose-galactose malabsorption
14. History of glucose-6-dehydrogenase deficiency
15. Participation in any other IMP trial at the time of consent
16. Previous pregnancy failure on hydroxychloroquine

## 6.3 Selection of Participants

Definitions used in the HYPATIA study:

- Screening: Time from consent to randomisation
- Baseline visit: Randomisation to treatment arm/Initial IMP dispensation
- Pre-pregnancy study visits: Visits between randomisation and achieved pregnancy
- Pregnancy study visits: Study visits on routine follow up visits

Potentially eligible women will be identified in routine outpatient clinics. Patients will receive full information about the HYPATIA study, and if they decide to take part written informed consent will be obtained. Patients will have as much time as they need to consider their participation. Eligibility will be assessed and provided all eligibility criteria are fulfilled, study randomization can be undertaken and the IMP can be dispensed (baseline visit) (see trial flow chart).

Once a woman is enrolled into the HYPATIA study, she will attend follow up visits every three months until she falls pregnant (at 3, 6, 9 and 12 months, defined as pre-pregnancy visits) in addition to her usual standard pregnancy care.
If no pregnancy is achieved in the 12 months from entering the study, this will be recorded in the study CRF and the study medication will be stopped. Once a woman has been enrolled into the HYPATIA study and has started taking the IMP, she will have a blood test including full blood count, kidney function and liver function every three months during the pre-pregnancy period and during pregnancy (these tests will coincide with her follow up visits) and are to ensure that it is safe to take the IMP (hydroxychloroquine).
On these visits the IMP will be repeatedly dispensed and compliance will be assessed. This will be documented in medical records and recorded in the eCRF.
When a woman falls pregnant she will contact the study team and will attend her first pregnancy visit (1$^{st}$ trimester visit). At this visit a new trial medication will be dispensed.
Follow up visits for the HYPATIA study purpose will be scheduled once every trimester (defined as 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ trimester visits), however, if the participant required closer monitoring she will be followed up accordingly as per standard of care. Women will be instructed to stop trial medication on the day of delivery and will be asked to hand in the rest of the trial medication at their post-partum follow up. The post-partum visit shall be completed 6 weeks post-partum. For some women it may be difficult to attend a follow up visit within 6 weeks after delivery (due to sleep deprivation or other challenges with a newborn) and the study teams can therefore contact the patient post-delivery to collect outcome data (date of delivery & information about the child). This can be decided on an individual basis.

All eligible women will have been diagnosed with persisting antiphospholipid antibodies (aPL) prior to trial entry. Results of aPL, i.e. anticardiolipin IgG and IgM antibodies, beta2glycoptroteinI IgG and IgM antibodies and lupus anticoagulant, confirming their presence or a diagnosis of APS. For the purpose of the HYPATIA study the last positive test must be within 12 months of study entry. The dates when the tests were performed routinely at the trial sites have to be documented in the notes and recorded in the eCRF.
Patients with anticardiolipin (IgG or IgM) antibodies and/or beta2glycoptroteinI (IgG or IgM) antibodies above the 95$^{th}$ percentile are considered positive and the levels will be recorded in the CRF. Each site will be asked about their local normal ranges (i.e. the local cut off for the 95$^{th}$ percentile) for anticardiolipin (IgG or IgM) antibodies and/or beta2glycoptroteinI (IgG or IgM) antibodies, which also have to be recorded in the patient notes and eCRF.
For the purpose of the HYPATIA study blood test results from prior to entering the trial can be used and for the purpose of the HYPATIA study with two specifications:

- the last positive aPL test result must be within 12 months of study entry
- Other blood results such as full blood count, kidney and liver function must be within 3 months of study entry.

We accept past positive antiphospholipid antibody screens as valid, as long as they have a positive screen within 12 months of study entry and if there is physical evidence of such in the patients' records.

## 6.4 Randomisation Procedure / Code Break

Written informed consent to enter the trial and to be randomized must be obtained from each patient, after explanation of the aims, methods, benefits and potential hazards of the trial and before any trial procedure is conducted.

It must be made completely and unambiguously clear that the patient 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.

Signed consent forms must be kept by the investigator and documented in the medical records and a copy given to the patient, with another copy filed in medical records. Upon consent, a letter should be sent to the general practitioner informing him/her of the trial and the participant's involvement in it.

Women with aPL are usually considered as having high-risk pregnancies and are monitored closely during pregnancy. For the purposes of HYPATIA study, we would expect patients to be seen for a minimum number of visits as indicated in section 4.2.

Women who decide to participate in the HYPATIA trial will have their usual care plus HCQ or placebo in addition to their usual medication.

Women recruited at selected centres will be invited to have a blood sample taken once at baseline, prior to pregnancy, once every trimester and post-partum for additional research. These samples will be analyzed for biomarkers and for pharmacokinetics of hydroxychloroquine in pregnancy.

Funding for the above studies will be obtained separately.

### 6.4.1 Randomisation

Randomisation will be undertaken by the local research team at each site once written informed consent has been obtained, eligibility confirmed, and baseline data collected.

Randomisation is by the method of minimisation, balanced by thrombosis (yes/no), previous adverse pregnancy outcomes (yes/no), previous pregnancy (yes/no), refractory obstetric APS (yes/no) and center.

Randomisation will be performed via a bespoke web-based randomisation system hosted by the King's College London, Clinical Trials Unit (KCTU). This system can be accessed 24 hours a day. Authorised site staff will be allocated a username and password for the system by the Trial Manager. If new staff members join the study, a user-specific username and password must be requested via the Trial Manager from the KCTU team and a request for access to be revoked must be requested when staff members leave the project. Study site staff experiencing issues with system access or functionality should contact the Trial Manager in the first instance.

Once eligibility has been confirmed by recruiting sites, authorised site staff will log into the system by going to www.ctu.co.uk and clicking the link to access the randomisation system. A full audit trail of data entry will be automatically date and time stamped, alongside information about the user making the entry within the system. Staff will enter key information about the patient, including the unique Participant Identification Number (PIN) generated from the Elsevier MACRO electronic data capture (EDC) system after consent. Once a patient is randomised, study drug will be automatically allocated in the system and confirmation emails will be sent to relevant personnel, with appropriate information.

If there are any difficulties in accessing this KCTU website, investigators should call the number below.

**https://ctu.co.uk**

**Telephone: + 45 60550372**
**(or e-mail: kschreiber@danskgigthospital.dk or rosa.caroline.jullie.rudnicki@regionh.dk> )**

The investigator, research team, site pharmacy team and the trial manager will be kept blind to the treatment allocation.

Unique pack numbers will be generated for the active and placebo products and will be sent with randomization emails. The trial statistician will know only A vs B, with the meaning of A & B held by the KCTU.

Detailed description of the randomization process: The IMP pack details will be uploaded to the randomization system so the system will know what the production pharmacy has available centrally.
For any site the trial manager will order the IMP packs as required. That will generate an email with specific pack numbers to be sent to site via the study manufacturing pharmacy at GSTT. Once the IMP has arrived at the requested study site, the trial manager will mark them as 'received'. Then, when a patient is randomized at that site, the system will automatically select a pack in the right trial arm and that pack number will be on the confirmation email, so it can be prescribed. When a patient comes in for follow up, the site will go into the system and allocate a new pack for them and the system will select one in the same trial arm.

### 6.4.2 Emergency Code Break

All participants will remain blind to treatment allocation until the primary analyses are complete and the primary paper has been accepted. Sites will be informed of the participants treatment allocation at this point.

Treating physicians should only request emergency code break when information about the participant's trial treatment is necessary for the appropriate medical management of the participant and where stopping the blinded medication is not sufficient. The treating physician or investigator will have the primary right to break the blind in any moment in case of emergency and they will be able to unblind immediately and without delay.

The HYPATIA Trial has commissioned a 24-hour telephone-based emergency code break service, Emergency Scientific Medical Services (eSMS). Participants will be asked to carry emergency cards during the study, which will have details of the code break telephone number. The code break number will also be printed on the study medication bottles. The caller can request code break from eSMS using either the IMP treatment bottle number or the participants study PIN, which is allocated by the MACRO eCRF system after consent.

ESMS will notify the Danish Sponsor of any code break requests received, irrespective of outcome. The Sponsor will inform the Principal Investigator of instances of unblinding. This will be recorded and the trial statistician informed at the analysis stage of the trial.

## 6.5 Withdrawal of Subjects

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

- drug intolerance
- any change in the patient's condition that justifies the discontinuation of the treatment in the clinician's opinion
- withdrawal of consent for treatment by the patient

As the patient's participation in the trial is entirely voluntary, they may choose to discontinue the trial treatment at any time without loss of benefits to which they are otherwise entitled. Although the patient is not required to give a reason for discontinuing their trial treatment, a reasonable effort should be made to establish this reason while fully respecting the patient's rights.

Patients who withdraw from the treatment will be asked to remain in the trial for the purpose of follow-up and data analysis (unless the patient withdraws their consent from all stages of the trial). If a patient decides to withdraw from the HYPATIA study no further data can be collected, however data collected until the point of withdrawal will be retained. If a patient decides to only withdraw from treatment, data and adverse events will be followed up. Adverse events will be followed up regardless of the IMP status until the end of the trial.
In the specific circumstance when a patient would like to withdraw from the trial, but allows us to collect the results of the pregnancy and data on the child from medical notes without contacting her, a consent form 2 has to be signed. In such case only the pregnancy outcome data will be collected following the withdrawal and adverse events will not be reported.

The investigator also has the right to withdraw patients from the study drug in the event of inter-current illness, adverse events (AEs), serious adverse events (SAE's), Suspected Unexpected Serious Adverse Reaction (SUSAR's), protocol violations, cure, administrative reasons or other reasons.

If the rare event occurred that women develop abnormal creatinine clearance of 30ml/min women will be withdrawn from the trial.

It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable; therefore, unnecessary withdrawal of patients should be avoided**.** Patients who withdraw from the study will not per se be replaced. However, this decision will be left to the discretion of the Data Monitoring Committee.

## 6.6 Expected Duration of Trial

The end of the trial is defined as database lock.

Each participant will remain in the study as long as their time to conception (up to maximum 12 months) and their full length of pregnancy until delivery (a total maximum of 21 months). In total a number of 328 pregnant patients will be included in the study. The total study duration is 108 months.

# 7 Trial Procedures

## 7.1 By Visit

The sequence of visits is outlined in the flowchart in section 4.3.

All patients participating in the HYPATIA trial will be seen pre-conception, then at least on a 3 monthly basis for medication refill until conception, and then once every trimester and post-partum according to a routine standard of care protocol (outlines in the assessment schedule outlined in section 4.3). This will enable face-to-face contact with patients to discuss any concerns. Patients will be advised to contact the research team to report any concerns about the treatment between trial visits.

| ***SCREENING VISIT*** | |
|---|---|
| • *Not pregnant yet, but is actively planning pregnancy*<br>• *The patient will be screened for eligibility*<br>• *Presence of antiphospholipid antibodies will be confirmed* | |
| **Pre-**pregnancy | • Written informed consent<br>• Eligibility assessment<br>• Blood results can be obtained from routine blood results within 12 months prior to entering the HYPATIA study. The following blood results will be reviewed & have to be recorded in the patient notes and eCRF:<br>  ○ **aPL antibodies including date of test, titers, units and reference range for 95$^{th}$ percentile used in local lab – repeat if necessary**<br>  ○ ENA antibodies<br>• Baseline bloods (FBC, renal function and liver function) as standard of care bloods before IMP start – can be obtained from routine blood results within 3 months prior to entering the study<br>• Demographic information (see options in 'Glossary of Terms")<br>• Height & weight<br>• Smoking status<br>• Physical exam (including lung and heart stethoscopy)<br>• Urine dip<br>• Blood pressure & Pulse<br>• Current medication<br>• Past medical and past pregnancy history<br>• eCRF information will be filled out |

Women will not be pregnant at this stage but are actively trying to fall pregnant. In case a woman is pregnant but not known to be pregnant at recruitment but who subsequently is confirmed pregnant within next LMP, the patient will remain in the trial and these data will analyzed as 'intention to treat'.
Potential participants will be consented if they are happy to participate in the HYPATIA study and will be assessed for eligibility. Patient notes (either in paper or electronic form) are defined as the source data and all study relevant information will be captured in the patient notes and then transferred into the eCRF.
Blood results from blood tests taken prior to entering the study can be used within the timelines specified below. If a patient has had a positive test for antiphospholipid antibodies prior to study entry within 12 months of study entry, these results can be used and recorded into the eCRF (test date and normal range have to be provided). For the screening routine blood test (full blood count, kidney and liver profile) pre-trial results not older than 3 months can be used. On an individual basis these blood results can be updated, but this will be left for the discretion of the treating physician.

**ONLY FOR WOMEN IN SELECTED CENTRES:** Women in selected centres will be invited to donate blood samples at baseline, once pre-pregnancy, every trimester and post-partum for further studies funded by separately. This will be outlined in the Laboratory section.

| ***BASELINE VISIT*** | |
|---|---|
| • *Confirmation of eligibility*<br>• *Randomization*<br>• *IMP dispensation* | |
| | • Adverse events monitoring<br>• Current medication<br>• Eligibility confirmation<br>Randomization & IMP dispensation – every 3 months<br><br>• From now the patient will be followed up on a three monthly basis if not pregnant & will be advised to contact the study team once pregnancy is confirmed. IMP will be dispensed on each of the 3-montly pre-pregnancy visits.<br><br>• Women will be instructed that they will be followed up on a 3 monthly basis from now. This is part of our standard of care protocol for women being treated at all centres and we expect our participating centers to adapt this approach for the purpose of the HYPATIA trial. Women will also be instructed to contact their local study team once they fall pregnancy and will then be seen by the local study team for review. This is standard of care in these women, and will coincide with the HYPATIA study visits.<br><br>• *Selected centres: bloods for additional research* |

| ***PRE-PREGNANCY VISITS (up to 4 visits)*** | |
|---|---|
| • *At this stage the patient is taking the IMP & is still actively planning to fall pregnant*<br>• *IMP re-dispensation*<br>• *Visits every 3 months until patient pregnant, max 4 times (a total of 12 months)* | |
| | • Current medication<br>• IMP dispensation<br>• Adverse events monitoring<br>• Compliance check (Kit number to be noted, date of current kit started & ended, number of tablets taken, number of tablets missed)<br>• Physical exam (including lung and heart stethoscopy)<br>• Blood test including full blood count, kidney and liver function to monitor for HCQ toxicity<br><br>• *Selected centres: bloods for additional research* |

Once a patient becomes pregnant, the patient will be brought back to clinic. The 'pregnancy progress' will be recorded in the patient notes.

| PREGNANCY follow up visit 1<br><br>1st Trimester visit (Gestational week 1 – 13+6) | |
|---|---|
| *The patient has been allocated to either hydroxychloroquine or placebo and is pregnant. At this stage patients will require new trial medication, which should be handed out at this visit. The reason to supply the patient with new trial medication is to ensure sufficient trial medication for follow up visits.* | |
| Pregnancy | Information on pregnancy will be collected:<br>• IVF pregnancy<br>• Singleton, Twin or Triplet pregnancy<br>• How many weeks has patient taken trial medication<br>• Current gestation<br>• Estimated Date of Delivery<br>• Physical exam (including lung and heart stethoscopy)<br><br>Blood test including full blood count, kidney and liver function to monitor for HCQ toxicity<br>*Selected centres: bloods for additional research* |
| Medication | • All medications will be recorded.<br>• Compliance check (Kit number to be noted, date of current kit started & ended, number of tablets taken, number of tablets missed)<br>• IMP dispensation<br><br>The patient will be asked to bring unused trial medication and empty packaging to each visit (number of tablets taken & number of tablets missed will be recorded), this will be collected, and a new supply will be given. |
| Adverse events & Adverse pregnancy events | Enquiry about adverse events including adverse pregnancy events. In case the patient has miscarried an 'adverse pregnancy events' form has to be filled out (not SAE). |

Participants who have fallen pregnant, will be seen on a regular basis in the pregnancy clinic. Information regarding the pregnancy will be documented in the patient notes and shall afterwards be transferred into the eCRF. In case a patient has had a miscarriage, information regarding the miscarriage will also be documented in the patient notes. The patient will be instructed to stop trial medication and to bring all spare trial medication to the next follow up visit. The remainder of the medication will then be counted and the results recorded in the CRF.

| PREGNANCY follow up visit 2<br><br>2nd Trimester visit (Gestational week 14 – 26+6) | |
|---|---|
| Pregnancy | Information on pregnancy will be collected:<br>• How many weeks has patient taken trial medication<br>• Current gestation<br>• Estimated Date of Delivery<br>• Physical exam (including lung and heart stethoscopy)<br>• Blood test including full blood count, kidney and liver function to monitor for HCQ toxicity |

| | |
|---|---|
| | *Selected centres: bloods for additional research*<br>Record whether patient has had anomaly scan, record fetal growth and uterine artery Doppler results (results can be recorded at 2nd or 3rd trimester visit). Record FGR. |
| Smoking status | • Smoking status update |
| US growth scans | Depending on the individual patient one or more US scans have been performed during the second trimester. All available growth scan results will be recorded. Information will be obtained from maternity records. |
| Medication | • All medications will be recorded.<br>• Compliance check (Kit number to be noted, date of current kit started & ended, number of tablets taken, number of tablets missed)<br>• IMP dispensation<br><br>The patient will be asked to bring unused trial medication and empty packaging to each visit (number of tablets taken & number of tablets missed will be recorded), this will be collected, and a new supply will be given.<br>All medications will be recorded. Compliance will be verified.<br>The patient will be asked to bring unused trial medication and empty packaging to each visit, this will be collected, and a new supply given every 3 month. |
| Adverse events & Adverse pregnancy events | Enquiry about adverse events including adverse pregnancy events. In case the patient has miscarried an 'adverse pregnancy events' form has to be filled out (not SAE). |

| **PREGNANCY follow up visit 3**<br><br>**3rd Trimester visit (Gestational week 27 – end of pregnancy)** | |
|---|---|
| Pregnancy | Information on pregnancy will be collected:<br>• How many weeks has patient taken trial medication<br>• Current gestation<br>• Estimated Date of Delivery<br>• Compliance check<br>• Physical exam (including lung and heart stethoscopy)<br><br>• Blood test including full blood count, kidney and liver function to monitor for HCQ toxicity<br><br>*Selected centres: bloods for additional research*<br>Record whether patient has had anomaly scan, record fetal growth and uterine artery Doppler results (Doppler results from 2nd trimester should also be recorded here). Record FGR. |
| US growth scans | Depending on the individual patient one or more US scans have been performed so far. All available growth scan results will be recorded. Information will be obtained from maternity records. If uterine artery Doppler results have not been documented at previous visit, they should be recorded. |

| | |
|---|---|
| Adverse events & Adverse pregnancy events | Enquiry about adverse events including adverse pregnancy events. In case the patient has miscarried an 'adverse pregnancy events' form has to be filled out (not SAE). |
| Medication | • All medications will be recorded.<br>• Compliance check (Kit number to be noted, date of current kit started & ended, number of tablets taken, number of tablets missed)<br>• IMP dispensation<br><br>The patient will be asked to bring unused trial medication and empty packaging to each visit (number of tablets taken & number of tablets missed will be recorded), this will be collected and a new supply will be given. |
| Post delivery | In case of preterm delivery, record actual date and mode of delivery, gestational age, gender, birth weight, Apgar score, neonatal morbidity (neonatal haemorrhage or thrombotic complications, infections, congenital abnormalities) neonatal days in special care, days to discharge from hospital following delivery (mother and child). |

| **POST PARTUM follow up visit**<br><br>**Post partum visit (up to 6 weeks post partum)** | |
|---|---|
| Mother | • None |
| Child | • Record actual date and mode of delivery<br>• Gestational age<br>• Child gender<br>• Birth weight<br>• Apgar score<br>• Neonatal morbidity (bleeding or thrombotic complications, infections, congenital abnormalities, days in special care)<br>• Days of admission (mother & child).<br><br>*Selected centres: bloods for additional research* |
| US growth scans | Depending on the individual patient one or more US scans have been performed during pregnancy. All available growth scan results will be recorded. Information will be obtained from maternity records. |
| Medication | • All medications will be recorded.<br>• Compliance check (Kit number to be noted, date of current kit started & ended, number of tablets taken, number of tablets missed)<br><br>The patient will be asked to bring unused trial medication and empty packaging (number of tablets taken & number of tablets missed will be recorded). |
| Adverse events & Adverse pregnancy events | • Enquiry about adverse events including adverse pregnancy events. In case the patient has had a miscarriage this will be noted in the patient notes and the eCRF (not SAE). |

## 7.2 Laboratory Tests

We will be using test results from standard of care lab for the study. These test results include, antibody profile (antiphospholipid antibodies, ENA).
If these blood tests never have been performed, they should be done as routine blood tests. Women will have a blood test including full blood count, kidney and liver function at screening (available pre-trial results from within 3 months can be used for study purposes) and every three months once they have started the IMP to ensure the monitoring of potential adverse side effects caused by hydroxychloroquine. If the rare event occurred that women develop abnormal creatinine clearance of 30ml/min women will be withdrawn from the trial.

*Laboratory tests for selected sites:*
Women will be invited to donate 15 ml of blood at baseline, at the first pre-pregnancy visit, once every trimester and post partum (max 6 weeks post partum). Only women recruited in Denmark will be invited to donate 17,5 ml of blood (as pax-gene tube will be included) at baseline, at the first pre-pregnancy visit, once every trimester and post partum (max 6 weeks post partum). Specimens will be analyzed for HCQ levels (pharmacokinetics) and biomarker studies. Where possible the blood collection will coincide with routine blood tests and study bloods will be kept locally in established laboratories. A research team member will personally transport the bloods to the laboratory. Blood samples can be handled in room temperature. These studies will be undertaken on separate funding. A log will be kept for the blood samples. This log will be signed be the research team member transporting the blood samples to the laboratory.

# 8 Assessment of Efficacy

## 8.1.1 Primary Efficacy Parameters

The primary efficacy parameters will include the absence of pregnancy complications, i.e. as defined in the primary endpoint is defined as follows:

The primary endpoint is a composite of three principal aPL-related adverse pregnancy outcomes: one or more pregnancy loss(es) (either < 10 weeks gestation or beyond 10 weeks of gestation of a morphologically normal fetus documented by ultrasound or by direct examination of the fetus) and premature birth of a morphologically normal neonate before 34 weeks due to any of: pre-eclampsia, eclampsia, recognized features of placental insufficiency
Premature birth for other reasons will not be included.

The components of the primary endpoint will each be presented as secondary endpoints (below).

The pre-defined secondary endpoints include:

1. Pregnancy loss < 10 weeks gestation
2. Pregnancy loss > 10$^{th}$ weeks of gestation of a morphologically normal fetus documented by ultrasound or by direct examination of the fetus
3. Premature birth of a morphologically normal neonate < 34 weeks due to any of: pre-eclampsia, eclampsia, recognized features of placental insufficiency.
4. Gestational age at delivery
5. Birth weight
6. Delivery by Caesarean section
7. Apgar score < 7 at 5 min
8. Neonatal morbidity (bleeding or thrombotic complications, infections, congenital abnormalities)
9. Days to hospital discharge following delivery (mother & child)
10. Thrombotic events in the mother during pregnancy and 6 weeks post-partum.
11. Days of neonate in special care
12. Safety and tolerability of hydroxychloroquine in the mother and in the neonate

The components of the primary endpoint will each be presented as secondary endpoints.

### 8.1.2 Secondary Efficacy Parameters

The secondary efficacy parameters will include the absence of the secondary endpoints as outlined above.

## 8.2 Procedures for Assessing Efficacy Parameters

The primary outcome of the HYPATIA study will be a composite of pregnancy complications as defined above (see primary and secondary outcomes).

Adverse pregnancy outcomes are defined under ‘Glossary and Definition’ (Section 2). Each adverse pregnancy outcome will be recorded for each individual case. Fetal growth will be assessed by using standardized ultrasound methods. Pre-eclampsia will be diagnosed according to international criteria (see definition in section 2), information with regards to the baby, i.e. gestational age at delivery, birth weight, mode of delivery, Apgar < 7 at 5 min, neonatal morbidity (bleeding or thrombotic complications, infections, congenital abnormalities), number of days in hospital and thrombotic events in the mother during the puerperium. Patients will be consented to give the trial team access to their child’s hospital notes if needed (i.e. in case of admission to paediatric special care units or complications such as intraventricular haemorrhage).

Symptoms suggestive of VTE will be evaluated according to the usual diagnostic methods. Patients who present with symptoms suggestive of stroke, myocardial infarction or arterial thrombosis at other sites, generally present as a clinical emergency and clinical and diagnostic information will be obtained retrospectively from the center at which they have been managed.

Efficacy makers are defined as follows:

*Miscarriage:* The presence of a miscarriage will be determined due to standard medical practice at each participating unit. Usually this will include a history from the patient and at some centers ultrasound for confirmation.

*Fetal death:* This will be determined by ultrasound according to standard medical practice.

*Intrauterine growth restriction:* Serial ultrasound assessments according to standard medical practice and the use of growth percentiles will be used to determine the presence or absence of fetal growth restriction (individualized birth weight according to Gardosi).

*Placental abruption:* Patient history and ultrasound according to standard medical practice.

*Premature birth:* Birth between 24+0 and 36+6 weeks gestation.

*Pre-eclampsia as defined in international guidelines* [4]: Hypertension developing after 20 weeks gestation and the coexistence of one or more of the following new onset conditions:

1. Proteinuria (spot urine protein/creatinine >30 mg/mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/L [‘2 + ’] on dipstick testing)
2. Other maternal organ dysfunction:
    - renal insufficiency (creatinine >90 umol/L)
    - liver involvement (elevated transaminases and/or severe right upper quadrant or epigastric pain)
    - neurological complications (examples include eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata)
    - haematological complications (thrombocytopenia, DIC, haemolysis)
3. Uteroplacental dysfunction
    - fetal growth restriction

# 9 Assessment of Safety

## 9.1 Specification, Timing and Recording of Safety Parameters.

The trial visit schedule will enable face-to-face contact with patients to discuss any concerns and to address any possible adverse events or side effects. Adverse events will be followed up until resolution.

Patients will be advised to contact their respective research team to report any concerns about the treatment between trial visits. All babies will be having an eye and ear test within 6 weeks post-partum.

Patients with APS planning for pregnancy or pregnant are seen with a close follow up. For pregnant patients with APS the usual scheduled visits at St Thomas’ Hospital, London are once every month, however, this practice may vary amongst centers. For the purpose of the HYPATIA study, we will expect all participating centers to adopt a similar approach.

Trial visits will be scheduled to coincide with routine follow up appointments and trial participation will not necessarily entail more frequent visits.

At each routine specialist follow-up clinic visit, routine clinical assessment will include assessment with regard to potential medication side effects. A blood test full blood count, kidney and liver function and a physical exam to ensure no potential adverse effects of hydroxychloroquine will be performed every three months as long as the patient is taking the IMP. These blood tests will be performed on the day of the usual visit, which means patients do not have to come to extra hospital appointments.

## 9.2 Procedures for Recording and Reporting Adverse Events

The Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 gives the following definitions:

**Adverse Event (AE):** Any untoward medical occurrence in a subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.

**Adverse Reaction (AR):** Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

**Unexpected Adverse Reaction** (UAR): An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the summary of the products characteristics (SmPC).

**Serious adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR)**: Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that

Results in death;
Is life-threatening;
Required hospitalization or prolongation of existing hospitalization;
Results in persistent or significant disability or incapacity;
Consists of a congenital anomaly or birth defect.

Fetal death as a result of maternal disease and other events that are primary or secondary outcome measures are not considered to be SAEs and should be reported in the normal way, on the appropriate CRF.

**Important Medical Events (IME):** Events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

**Reporting Responsibilities**

Rigshospitalet, Denmark 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 GCP unit in Copenhagen.

All SAEs, SARs and SUSARs (excepting those specified in this protocol as not requiring reporting) will be reported immediately (and certainly no later than 24hrs) by the Investigator to the KHP-CTO and CI for review in accordance with the current Pharmacovigilance Policy.

The GCP unit in Copenhagen will report SUSARs to the relevant regulatory authorities (MHRA in the UK, competent authorities of other EEA (European Economic Area) states in which the trial is taking place.

The Chief Investigator will report to the relevant ethics committee. Reporting timelines are as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.
- The Chief Investigator and GCP unit in Copenhagen (on behalf of the sponsor), will submit a Development Safety Update Report (DSUR) relating to this trial IMP, to the MHRA and REC annually.

### 9.2.1 Adverse events that do not require reporting

Pregnancy itself is well known to potentially causing nausea and tiredness due to several physiological adjustments of the body especially in the first trimester. Any symptoms thought to be related to the physiological changes in pregnancy do not require reporting.

In patients with aPL we expect pregnancy complications. Therefore, aPL related pregnancy complications, such as miscarriage < 10 weeks gestation, miscarriage > weeks 10, premature birth < 34 weeks due to pre-eclampsia, eclampsia or FGR and stillbirth shall not be reported as SAE. For these events we have designed a special section in the electronic database 'adverse pregnancy outcomes', which shall be completed in this case. Any other adverse events will be reported.

## 9.3 Treatment Stopping Rules

The trial may be prematurely discontinued by the Sponsor, Chief Investigator or Regulatory Authority on the basis of new safety information or for other reasons given by the Data Monitoring & Ethics Committee / Trial Steering Committee regulatory authority or ethics committee concerned.

If the trial is prematurely discontinued, active participants will be informed and no further participant data will be collected. The Competent Authority and Research Ethics Committee will be informed within 15 days of the early termination of the trial.

# 10 Statistics

## 10.1 Analysis

Primary analysis

Percentages in both arms of the study will be compared using binomial regression with a log link, adjusting for the minimization variables to give risk ratios. Risk differences will be estimated similarly. Continuous measures will be compared using linear regression adjusting for minimization variables and baseline measurement of the outcome (where available). Estimates will be given with 95% confidence intervals.

Results will be considered significant at the 5% level. However, attention will be paid to the totality of results and the exact size of the P-value. Smaller values will be treated as stronger evidence against the null hypothesis.

The intention-to-treat principle will be followed in the main analysis, but a secondary per-protocol analysis will also be performed to provide additional information on the nature of the treatment effect.

Subgroup analysis

Subgroup analyses in RCTs mainly serve to investigate possible moderators of the treatment effect – i.e. subgroups of women identifiable at trial entry who will do particularly well or badly with the treatment in question.

Subgroup analyses will only be carried out where there are at least 20 women in each subgroup. Subgroup analyses will be carried out for all minimisation variables.
Two further subgroups analyses are planned, to investigate whether it is possible to identify groups of women for whom hydroxychloroquine would be more or less effective. The first analysis will investigate the way in which aPL was first identified: random testing, recurrent miscarriage, fertility clinic (IVF) or thrombosis. The second will be according to their aPL titre at trial entry: i.e. is it low titre (>95th percentile) alone or according to the Sydney criteria[1].

In each case, the main treatment effect will be assessed in all subgroups, and interaction tests carried out to determine if treatment effect differs significantly between subgroups.

Pharmacokinetics and biomarkers will be investigated in women at St Thomas' only; and will form the focus of a separate study.

Additional points:

- Description of the statistical methods employed – including time(s) for scheduled interim analyses

  Statistical methods are described above.

  No formal interim analyses are planned. The Data Monitoring Committee will aim to meet in person at least every 6 months, and will consider the results so far. They are empowered to request that the trial stops if there is overwhelming evidence for or against one treatment are such that to randomize further patients would be unethical.

- Procedures for dealing with missing data, unused data and false data. False data may be e.g. interpolated data, and it is not necessarily false

  We will follow a four point framework for dealing with incomplete observations which will allow the correct method to be chosen and subsequently implemented.
  1. Attempt to follow up all randomized participants, even if they withdraw from allocated treatment
  2. Perform a main analysis of all observed data that is valid under a plausible assumption about the missing data. Specifically, we will assume data is missing at random (MAR). Under this assumption, imbalances between treatment groups due to dropout can be corrected by appropriate multiple regression models.
  3. Perform a sensitivity analyses to explore the effect of departures from the assumption made in the main analysis. The MNAR (missing not at random) analysis will use the method of White et al. (2011) [28] as implemented in the Stata command rctmiss.
  4. Account for all randomized participants, at least in the sensitivity analyses

  This framework highlights the importance of using plausible assumptions with regards to the nature of the missing data. These assumptions will then be tested using appropriate sensitivity analyses on observed data using complete cases analysis. For the purpose of the main analysis we will make the assumption that missing data is missing at random and the effect of the intervention is the same in those with and without the observations. Furthermore, checks will be made to ensure that the percentage of missing data within each treatment allocation is not significantly different.

  We do not intend to create false data. No duly randomized subjects with outcome data will be excluded. All relevant data collected will be used.

- Procedures for reporting deviations from the original statistical plan

  It is not intended to deviate from the data analysis plan as agreed; but if it should prove necessary, the nature, reasons and implications of such deviations for interpreting the results, will be reported to the funders and in any subsequent publications.

- Statement of the trial subjects, whose data will be included in the statistical analysis (e.g. all randomised subjects, all subjects receiving medication, all eligible subjects and all subjects who can be evaluated).

The principal analysis will be according to the intention-to-treat principle, reporting all subjects with analyzable data in their original randomized groups.

## 10.2 Sample Size

The sample size was calculated based on our audit data.

Retrospective data from St Thomas' Hospital showed that treatment with HCQ was associated with a higher rate of live birth (67% in women with SLE and aPL treated with HCQ versus 57% in women in the control group p=0.005) and a lower prevalence of aPL related pregnancy morbidity (47% versus 63% p=0.004).

The HCQ intervention will be added to current standard of care. The trial is powered to detect a 16% reduction in pregnancy morbidity, which is the main outcome of the study (total sample size 328). A minimum of 328 women will therefore be randomized. In order to achieve a number of 328 pregnant women, we will randomize 400 patients. This takes into account those women who do not achieve pregnancy within 12 months and those who do not pregnant due to other reasons.

The calculation was made on the following: significance alpha = 0.05, power 1-beta 80%, percentage cross 5%.

# 11 Trial Steering Committee

A HYPATIA steering committee has been appointed. The committee includes Prof Søren Jacobsen, Prof Hunt, Dr Schreiber, further HYPATIA study team members and a lay person. A dedicated person from the Clinical Trials Unit will be appointed. An independent chair will be appointed.
The overall role will be to provide overall supervision of the HYPATIA study and the steering committee will meet on a 6 monthly basis. The Steering Committee is the main decision making body. It has overall responsibility for scientific strategy and direction and has ultimate responsibility for ensuring the project's aims are delivered on time and within budget.

# 12 Data Monitoring Committee

A Data Monitoring Committee has been appointed. The committee includes an independent chair, an independent statistician, the trial statistician and an independent expert.
The DMC has decided the following stopping rules:

Confirmed option of unblinding available at any stage, BUT only if concern that treatment will clearly harm or benefit patients

The simple rule of 3 standard errors will be applied to difference between groups.

# 13 Direct Access to Source Data and Documents

The Investigator(s) will permit trial-related monitoring, audits, REC review, and regulatory inspections by providing the Sponsor(s), Regulators and REC direct access to source data and other documents (e.g. patients' case sheets, blood test reports, X-ray reports, histology reports etc.).

# 14 Ethics & Regulatory Approvals

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments and applicable local regulations in participating countries.

This protocol and related documents will be submitted for review to the South London Research Ethics Committee (REC), and to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorization in the UK and to respective bodies in all participating countries.

The Chief Investigator will submit a final report at conclusion of the trial to the KHP-CTO (on behalf of the Sponsor), the REC and the MHRA (regulatory authorities of other states in which the trial is taking place) within the timelines defined in the Regulations.

# 15 Quality Assurance

Monitoring of this trial to ensure compliance with Good Clinical Practice and scientific integrity will be managed and oversight retained by the Good Clinical Practice (GCP) Unit (Copenhagen) and a dedicated monitor.

# 16 Data Handling

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

Patient data will be anonymized.

- All anonymized data will be stored on a password-protected computer.
- All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the Kings Health Partners Clinical Trials Office Archiving SOP. For our EU sites: All trial data will be stored and archived according to applicable local regulatory requirements.

For blood samples obtained from women at St Thomas' Hospital/UK:

Samples will be stored linked anonymized (i.e. using participant study number) in locked freezers in the Thrombosis Research laboratory, St Thomas' Hospital.
Only members of the research team have access to the samples. All laboratories are locked by the end of the day and freezers are also constantly locked. Only members of the research team have access. After 15 years of storage blood samples will be discarded.

For samples obtained from women at Danish, Irish and Canadian study sites:
Samples will be stored linked anonymized (i.e. using participant study number) in locked freezers at each participating sites locally. After 25 years of storage blood samples will be discarded.

# 17 Data Management

A website for the HYPATIA trial will be created. All participating investigators will be given an individual access to the website in order to enter data.

A MACRO database is provided by the KCTU. Information about the patient will be kept in the in medical records and will then be transcribed into the electronic CRF (eCRF). Each patient will be allocated a participant number (which will include the study site identification and the patient number) that will be used to identify the patient, no personal data will be recorded in the database. Data in the eCRF will be filled in at each trial visit.

A separate data management plan will be created for the trial.

# 18 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.

# 19 Insurance / Indemnity

The sponsor will provide indemnity for all NHS based sites in the UK, for sites in the EU and Canada.

# 20 Financial Aspects

Funding to conduct the trial is provided by the NIHR Research for Patient Benefit and the NOVO Nordisk Foundation.

# 21 Signatures

*To be signed by Chief Investigator minimum.*

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Chief Investigator Date

*Print name*

REFERENCES:


1. Miyakis, S., et al., *International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS).* J Thromb Haemost, 2006. **4**(2): p. 295-306.
2. Gardosi, J., et al., *Customised antenatal growth charts.* Lancet, 1992. **339**(8788): p. 283-7.
3. Gardosi, J., et al., *An adjustable fetal weight standard.* Ultrasound Obstet Gynecol, 1995. **6**(3): p. 168-74.
4. Tranquilli, A.L., et al., *The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP.* Pregnancy Hypertens, 2014. **4**(2): p. 97-104.
5. Parke, A.L., *Antimalarial drugs, systemic lupus erythematosus and pregnancy.* J Rheumatol, 1988. **15**(4): p. 607-10.
6. Parke, A., *Antimalarial drugs and pregnancy.* Am J Med, 1988. **85**(4A): p. 30-3.
7. Ostensen, M., et al., *Anti-inflammatory and immunosuppressive drugs and reproduction.* Arthritis Res Ther, 2006. **8**(3): p. 209.
8. Cervera, R., et al., *Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients.* Ann Rheum Dis, 2015. **74**(6): p. 1011-8.
9. Flint, J., et al., *BSR and BHPR guideline on prescribing drugs in pregnancy and breastfeeding-Part I: standard and biologic disease modifying anti-rheumatic drugs and corticosteroids.* Rheumatology (Oxford), 2016.
10. Andreoli, L., et al., *EULAR recommendations for women's health and the management of family planning, assisted reproduction, pregnancy and menopause in patients with systemic lupus erythematosus and/or antiphospholipid syndrome.* Ann Rheum Dis, 2017. **76**(3): p. 476-485.
11. Wallace, D.J., *The history of antimalarials.* Lupus, 1996. **5 Suppl 1**: p. S2-3.
12. Wallace, D.J., et al., *New insights into mechanisms of therapeutic effects of antimalarial agents in SLE.* Nat Rev Rheumatol, 2012. **8**(9): p. 522-33.
13. Pierangeli, S.S., M. Vega-Ostertag, and E.N. Harris, *Intracellular signaling triggered by antiphospholipid antibodies in platelets and endothelial cells: a pathway to targeted therapies.* Thromb Res, 2004. **114**(5-6): p. 467-76.
14. Rand, J.H., et al., *Hydroxychloroquine protects the annexin A5 anticoagulant shield from disruption by antiphospholipid antibodies: evidence for a novel effect for an old antimalarial drug.* Blood, 2010. **115**(11): p. 2292-9.
15. Giannakopoulos, B. and S.A. Krilis, *The pathogenesis of the antiphospholipid syndrome.* N Engl J Med, 2013. **368**(11): p. 1033-44.
16. Sorice, M., et al., *Anti-beta2-glycoprotein I antibodies induce monocyte release of tumor necrosis factor alpha and tissue factor by signal transduction pathways involving lipid rafts.* Arthritis Rheum, 2007. **56**(8): p. 2687-97.
17. Lopez-Pedrera, C., et al., *Antiphospholipid antibodies from patients with the antiphospholipid syndrome induce monocyte tissue factor expression through the simultaneous activation of*

*NF-kappaB/Rel proteins via the p38 mitogen-activated protein kinase pathway, and of the MEK-1/ERK pathway.* Arthritis Rheum, 2006. **54**(1): p. 301-11.
18. Ritis, K., et al., *A novel C5a receptor-tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways.* J Immunol, 2006. **177**(7): p. 4794-802.
19. Vega-Ostertag, M., et al., *Involvement of p38 MAPK in the up-regulation of tissue factor on endothelial cells by antiphospholipid antibodies.* Arthritis Rheum, 2005. **52**(5): p. 1545-54.
20. Wolberg, A.S. and R.A. Roubey, *Mechanisms of autoantibody-induced monocyte tissue factor expression.* Thromb Res, 2004. **114**(5-6): p. 391-6.
21. Kornberg, A., et al., *Induction of tissue factor-like activity in monocytes by anti-cardiolipin antibodies.* J Immunol, 1994. **153**(3): p. 1328-32.
22. Reverter, J.C., et al., *Effects of human monoclonal anticardiolipin antibodies on platelet function and on tissue factor expression on monocytes.* Arthritis Rheum, 1998. **41**(8): p. 1420-7.
23. Girardi, G., et al., *Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome.* J Clin Invest, 2003. **112**(11): p. 1644-54.
24. Schreiber, K., et al., *The effect of hydroxychloroquine on haemostasis, complement, inflammation and angiogenesis in patients with antiphospholipid antibodies.* Rheumatology (Oxford), 2018. **57**(1): p. 120-124.
25. Edwards, M.H., et al., *Hydroxychloroquine reverses thrombogenic properties of antiphospholipid antibodies in mice.* Circulation, 1997. **96**(12): p. 4380-4.
26. Mulla, M.J., et al., *Antiphospholipid antibodies limit trophoblast migration by reducing IL-6 production and STAT3 activity.* Am J Reprod Immunol, 2010. **63**(5): p. 339-48.
27. Pierangeli, S.S., et al., *Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia.* Arthritis Rheum, 2005. **52**(7): p. 2120-4.
28. Fischetti, F., et al., *Thrombus formation induced by antibodies to beta2-glycoprotein I is complement dependent and requires a priming factor.* Blood, 2005. **106**(7): p. 2340-6.
29. Meroni, P.L., et al., *Pathogenesis of antiphospholipid syndrome: understanding the antibodies.* Nat Rev Rheumatol, 2011. **7**(6): p. 330-9.
30. Breen, K.A.S., P.; Parmar, K.; Moore, G. W.; Stuart-Smith, S. E.; Hunt, B. J., *Complement activation in patients with isolated antiphospholipid antibodies or primary antiphospholipid syndrome.* Thrombosis and Haemostasis, 2012. **107**(3): p. 423-429.
31. Oku, K., et al., *Essential role of the p38 mitogen-activated protein kinase pathway in tissue factor gene expression mediated by the phosphatidylserine-dependent antiprothrombin antibody.* Rheumatology (Oxford), 2013. **52**(10): p. 1775-84.
32. Holers, V.M., et al., *Complement C3 activation is required for antiphospholipid antibody-induced fetal loss.* J Exp Med, 2002. **195**(2): p. 211-20.
33. Bertolaccini, M.L., et al., *Complement inhibition by hydroxychloroquine prevents placental and fetal brain abnormalities in antiphospholipid syndrome.* J Autoimmun, 2016.
34. Prinz, N., et al., *Endosomal NADPH-oxidase is critical for induction of the tissue factor gene in monocytes and endothelial cells. Lessons from the antiphospholipid syndrome.* Thromb Haemost, 2013. **109**(3): p. 525-31.
35. Muller-Calleja, N., et al., *Cofactor-independent antiphospholipid antibodies activate the NLRP3-inflammasome via endosomal NADPH-oxidase: implications for the antiphospholipid syndrome.* Thromb Haemost, 2015. **113**(5): p. 1071-83.
36. Muller-Calleja, N., et al., *Hydroxychloroquine inhibits proinflammatory signalling pathways by targeting endosomal NADPH oxidase.* Ann Rheum Dis, 2016.
37. Molad, Y., et al., *Protective effect of hydroxychloroquine in systemic lupus erythematosus. Prospective long-term study of an Israeli cohort.* Lupus, 2002. **11**(6): p. 356-61.
38. Ruiz-Irastorza, G., et al., *Effect of antimalarials on thrombosis and survival in patients with systemic lupus erythematosus.* Lupus, 2006. **15**(9): p. 577-83.
39. Fessler, B.J., et al., *Systemic lupus erythematosus in three ethnic groups: XVI. Association of hydroxychloroquine use with reduced risk of damage accrual.* Arthritis Rheum, 2005. **52**(5): p. 1473-80.
40. Schmidt-Tanguy, A., et al., *Antithrombotic effects of hydroxychloroquine in primary antiphospholipid syndrome patients.* J Thromb Haemost, 2013. **11**(10): p. 1927-9.
41. Mekinian, A., et al., *The efficacy of hydroxychloroquine for obstetrical outcome in anti-phospholipid syndrome: Data from a European multicenter retrospective study.* Autoimmun Rev, 2015. **14**(6): p. 498-502.
42. Sciascia, S., et al., *The impact of hydroxychloroquine treatment on pregnancy outcome in women with antiphospholipid antibodies.* Am J Obstet Gynecol, 2016. **214**(2): p. 273 e1-8.

43. Sciascia, S., et al., *The efficacy of hydroxychloroquine in altering pregnancy outcome in women with antiphospholipid antibodies. Evidence and clinical judgment.* Thromb Haemost, 2016. **115**(2): p. 285-90.
44. Flint, J., et al., *BSR and BHPR guideline on prescribing drugs in pregnancy and breastfeeding-Part II: analgesics and other drugs used in rheumatology practice.* Rheumatology (Oxford), 2016.
45. Schreiber, K., et al., *British Society for Rheumatology guideline on prescribing drugs in pregnancy and breastfeeding: comorbidity medications used in rheumatology practice.* Rheumatology, 2022.
46. Russell, M.D., et al., *British Society for Rheumatology guideline on prescribing drugs in pregnancy and breastfeeding: immunomodulatory anti-rheumatic drugs and corticosteroids.* Rheumatology, 2022.
47. Gotestam Skorpen, C., et al., *The EULAR points to consider for use of antirheumatic drugs before pregnancy, and during pregnancy and lactation.* Ann Rheum Dis, 2016. **75**(5): p. 795-810.
48. Levy, G.D., et al., *Incidence of hydroxychloroquine retinopathy in 1,207 patients in a large multicenter outpatient practice.* Arthritis Rheum, 1997. **40**(8): p. 1482-6.
49. Costedoat-Chalumeau, N., et al., *A Critical Review of the Effects of Hydroxychloroquine and Chloroquine on the Eye.* Clin Rev Allergy Immunol, 2015.
50. Marmor, M.F., et al., *Recommendations on Screening for Chloroquine and Hydroxychloroquine Retinopathy (2016 Revision).* Ophthalmology, 2016. **123**(6): p. 1386-94.
51. Ostensen, M.A., L.; Brucato, A.; Cetin, I.; Chambers, C.; Clowse, M. E. B.; Costedoat-Chalumeau, N.; Cutolo, M.; Dolhain, R.; Fenstad, M. H.; Forger, F.; Wahren-Herlenius, M.; Ruiz-Irastorza, G.; Koksvik, H.; Nelson-Piercy, C.; Shoenfeld, Y.; Tincani, A.; Villiger, P. M.; Wallenius, M.; von Wolff, M., *State of the art: Reproduction and pregnancy in rheumatic diseases.* Autoimmunity Reviews, 2015. **14**(5): p. 376-386.
52. Ruiz-Irastorza, G., et al., *Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systematic review.* Ann Rheum Dis, 2010. **69**(1): p. 20-8.
53. Sperber, K., et al., *Systematic review of hydroxychloroquine use in pregnant patients with autoimmune diseases.* Pediatr Rheumatol Online J, 2009. **7**: p. 9.
54. Izmirly, P.M., et al., *Evaluation of the risk of anti-SSA/Ro-SSB/La antibody-associated cardiac manifestations of neonatal lupus in fetuses of mothers with systemic lupus erythematosus exposed to hydroxychloroquine.* Ann Rheum Dis, 2010. **69**(10): p. 1827-30.
55. Motta, M., et al., *Immune system development in infants born to mothers with autoimmune disease, exposed in utero to immunosuppressive agents.* Am J Perinatol, 2007. **24**(8): p. 441-7.
56. Cimaz, R., et al., *Alterations in the immune system of children from mothers treated with immunosuppressive agents during pregnancy.* Toxicol Lett, 2004. **149**(1-3): p. 155-62.
57. Colvin, L., et al., *Linking a pharmaceutical claims database with a birth defects registry to investigate birth defect rates of suspected teratogens.* Pharmacoepidemiol Drug Saf, 2010. **19**(11): p. 1137-50.
58. de Man, Y.A., et al., *Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: results of a national prospective study.* Arthritis Rheum, 2009. **60**(11): p. 3196-206.
59. Diav-Citrin, O., et al., *Pregnancy outcome following in utero exposure to hydroxychloroquine: a prospective comparative observational study.* Reprod Toxicol, 2013. **39**: p. 58-62.
60. Friedman, D.M., et al., *Evaluation of fetuses in a study of intravenous immunoglobulin as preventive therapy for congenital heart block: Results of a multicenter, prospective, open-label clinical trial.* Arthritis Rheum, 2010. **62**(4): p. 1138-46.
61. Izmirly, P.M., et al., *Maternal use of hydroxychloroquine is associated with a reduced risk of recurrent anti-SSA/Ro-antibody-associated cardiac manifestations of neonatal lupus.* Circulation, 2012. **126**(1): p. 76-82.
62. Kuriya, B., et al., *Patterns of medication use during pregnancy in rheumatoid arthritis.* Arthritis Care Res (Hoboken), 2011. **63**(5): p. 721-8.
63. Motta, M., et al., *Follow-up of infants exposed to hydroxychloroquine given to mothers during pregnancy and lactation.* J Perinatol, 2005. **25**(2): p. 86-9.
64. Viktil, K.K., A. Engeland, and K. Furu, *Outcomes after anti-rheumatic drug use before and during pregnancy: a cohort study among 150,000 pregnant women and expectant fathers.* Scand J Rheumatol, 2012. **41**(3): p. 196-201.

65. Lockshin, M.D., et al., *Prediction of adverse pregnancy outcome by the presence of lupus anticoagulant, but not anticardiolipin antibody, in patients with antiphospholipid antibodies.* Arthritis Rheum, 2012. **64**(7): p. 2311-8.
66. Cimaz, R., et al., *Response to tetanus vaccination in infants exposed in utero to immunosuppressants for maternal autoimmune disorders.* Lupus, 2007. **16**(2): p. 129-32.
67. Losada, I., et al., *Bisphosphonates in patients with autoimmune rheumatic diseases: Can they be used in women of childbearing age?* Autoimmun Rev, 2010. **9**(8): p. 547-52.
68. Renault, F., et al., *Visual neurophysiological dysfunction in infants exposed to hydroxychloroquine in utero.* Acta Paediatr, 2009. **98**(9): p. 1500-3.
69. Anderka, M.T., et al., *Reviewing the evidence for mycophenolate mofetil as a new teratogen: case report and review of the literature.* Am J Med Genet A, 2009. **149A**(6): p. 1241-8.
70. David, A.L., et al., *Congenital fetal heart block: a potential therapeutic role for intravenous immunoglobulin.* Obstet Gynecol, 2010. **116 Suppl 2**: p. 543-7.
71. El Sebaaly, Z., B. Charpentier, and R. Snanoudj, *Fetal malformations associated with mycophenolate mofetil for lupus nephritis.* Nephrol Dial Transplant, 2007. **22**(9): p. 2722.
72. Huang, S.Y., et al., *Sonographic diagnosis of fetal malformations associated with mycophenolate mofetil exposure in utero.* Am J Obstet Gynecol, 2008. **199**(2): p. e6-8.
73. Streit, M., et al., *Successful pregnancy in pulmonary arterial hypertension associated with systemic lupus erythematosus: a case report.* J Med Case Rep, 2009. **3**: p. 7255.
74. Guo, Y.Y., et al., *Coexisting ankylosing spondylitis and rheumatoid arthritis: a case report with literature review.* Chin Med J (Engl), 2011. **124**(20): p. 3430-2.
75. Klinger, G., et al., *Ocular toxicity and antenatal exposure to chloroquine or hydroxychloroquine for rheumatic diseases.* Lancet, 2001. **358**(9284): p. 813-4.
76. Motta, M.B., M. C.; Tincani, A.; Avcin, T.; De Carolis, S.; Lachassinne, E., *Follow-up of babies born to mothers with antiphospholipid syndrome: preliminary data from the European neonatal registry.* Lupus, 2012. **21**(7): p. 761-3.
77. Cimaz, R., et al., *Electroretinograms of children born to mothers treated with hydroxychloroquine during pregnancy and breast-feeding: comment on the article by Costedoat-Chalumeau et al.* Arthritis Rheum, 2004. **50**(9): p. 3056-7; author reply 3057-8.
78. Costedoat-Chalumeau, N., et al., *Safety of hydroxychloroquine in pregnant patients with connective tissue diseases: a study of one hundred thirty-three cases compared with a control group.* Arthritis Rheum, 2003. **48**(11): p. 3207-11.
79. Rainsford, K.D., et al., *Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases.* Inflammopharmacology, 2015. **23**(5): p. 231-69.
80. Costedoat-Chalumeau, N., et al., *Evidence of transplacental passage of hydroxychloroquine in humans.* Arthritis Rheum, 2002. **46**(4): p. 1123-4.
81. Ding, H.J., et al., *Hydroxychloroquine-related retinal toxicity.* Rheumatology (Oxford), 2016. **55**(6): p. 957-67.
82. Wolfe, F. and M.F. Marmor, *Rates and predictors of hydroxychloroquine retinal toxicity in patients with rheumatoid arthritis and systemic lupus erythematosus.* Arthritis Care Res (Hoboken), 2010. **62**(6): p. 775-84.
83. Mackenzie, A.H., *Dose refinements in long-term therapy of rheumatoid arthritis with antimalarials.* Am J Med, 1983. **75**(1A): p. 40-5.
84. Wang, C., et al., *Discontinuation of antimalarial drugs in systemic lupus erythematosus.* J Rheumatol, 1999. **26**(4): p. 808-15.
85. Melles, R.B. and M.F. Marmor, *The risk of toxic retinopathy in patients on long-term hydroxychloroquine therapy.* JAMA Ophthalmol, 2014. **132**(12): p. 1453-60.
86. Mavrikakis, I., et al., *The incidence of irreversible retinal toxicity in patients treated with hydroxychloroquine: a reappraisal.* Ophthalmology, 2003. **110**(7): p. 1321-6.
87. Costedoat-Chalumeau, N., et al., *Heart conduction disorders related to antimalarials toxicity: an analysis of electrocardiograms in 85 patients treated with hydroxychloroquine for connective tissue diseases.* Rheumatology (Oxford), 2007. **46**(5): p. 808-10.
88. Sammaritano, L.R., et al., *2020 American College of Rheumatology guideline for the management of reproductive health in rheumatic and musculoskeletal diseases.* Arthritis care & research, 2020. **72**(4): p. 461-488.
89. Yusuf, I.H., B. Foot, and A.J. Lotery, *The Royal College of Ophthalmologists recommendations on monitoring for hydroxychloroquine and chloroquine users in the United Kingdom (2020 revision): executive summary*. 2021, Nature Publishing Group. p. 1532-1537.