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Hydroxychloroquine for Prevention of Recurrent Miscarriage: Study Protocol for a Multicenter Randomized Placebo Controlled Trial

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Hydroxychloroquine for Prevention of Recurrent Miscarriage:

Study Protocol for a Multicenter Randomized Placebo Controlled Trial

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ABSTRACT

Introduction Recurrent miscarriage (RM), defined by ≥ 3 consecutive losses during the first trimester of pregnancy, affects 1 to 2% of fertile couples. Standard investigations fail to reveal any apparent cause in ~ 50% of couples. However, on the basis of animal models and clinical studies, several hypotheses have been put forward concerning underlying mechanisms of RM: altered ovarian reserve, progesterone defect, thrombotic and/or endothelial dysfunction, immunological disturbances. Nonetheless, no study has yet reached conclusive beneficial clinical evidence for a potential treatment in unexplained RM. Hydroxychloroquine (HCQ) is a molecule with extensive safety data during pregnancy. HCQ pharmacological properties (e.g. anti-thrombotic, vascular protective, immunomodulatory, improved glucose tolerance, lipidlowering, antiinfectious) could be effective against some mechanisms of unexplained RM. Furthermore, eventhough clinical benefit of HCQ is suggested in prevention of thrombotic and late obstetric events in antiphospholipid (APL) syndrome, there is no data suggesting the benefit of HCQ in RM in the presence of APL antibodies.

Methods and analysis Taken all together and given the low cost of HCQ, the aim of this multicenter, randomized placebo-controlled, double-blind study is to investigate whether HCQ would improve the live-birth rate in women with RM, irrespective of maternal thrombophilic status: i) no known thrombophilia, ii) inherited thrombophilia or iii) APL antibodies. The primary end-point is a live and viable birth. After confirming eligibility and obtaining consent, 300 non-pregnant women will be randomized in 2 parallel groups for a daily oral treatment (HCQ 400 mg or placebo), initiated before conception and stopped at 10 weeks' gestation. If pregnancy doesn't occur after one year, the treatment will be stopped.

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3 **Ethics and dissemination** Agreement from the French National Public Health and Drug
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5 Security Agency (160765A-22) and ethical approval from the Committee for the Protection of
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7 Persons of NORD-OUEST I (2016-001330-97) have been obtained.
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10 **Trial registration number** ClinicalTrials.gov: NCT0316513
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STRENGTHS AND LIMITATIONS OF THIS STUDY

- Despite numerous fundamental research publications and clinical trials, the only recommendation that applies to follow-up of child bearing women suffering from RM relies on cocooning.
- This is the first randomized placebo-controlled study which aims to investigate whether oral hydroxychloroquine would improve the live-birth rate in women with recurrent miscarriage irrespective of maternal thrombophilic status.
- As a possible limitation, this study is based on the hypothesis that recurrent miscarriage often results from combined failures which could benefit from the pleiotropic effects of hydroxychloroquine.
- As hydroxychloroquine should probably be administered at least 2 menstrual cycles before conception to have an effect, women will be advised not to get pregnant during this period, but we do not plan to exclude those who will conceive too early.

INTRODUCTION

Background

RM is a common public health reproductive burden causing both physical and emotional distress. To date there is no treatment whose benefit has been clearly proved, even in the presence of well-known risk factors of RM. Most importantly, the absence of benefit has been clearly demonstrated for some treatments that are yet empirically proposed.

Unexplained Recurrent Miscarriage (RM)

A sporadic miscarriage is clinically detected in approximately 10% to 15% of pregnancies. Fetal development usually stops before 10 weeks.¹ Given the recurrent miscarriage (RM) frequency among fertile couples (1% to 2%) being significantly higher than the expected random one (153 = 0.34 %), RM is most often defined as ≥ 3 consecutive losses. Apart from the detection of a lethal chromosomal abnormality on products of conception, the underlying mechanism of loss remains unknown in most cases. The rate of normal embryonic karyotypes in RM steadily increases from the third loss, suggesting alternative mechanisms than meiotic aberrations.² Standard investigations fail to reveal any apparent cause in ~50% of the women. However, On the basis of animal models and clinical studies, several hypotheses have been put forward. Here, we focus on thrombosis and both endothelial and immune dysfunctions. Those could be targeted by pharmacological properties of hydroxychloroquine (HCQ).

An association with some inherited thrombophilia has been reported (factor V Leiden, mutation G20210A of the prothrombin and protein S deficiency), although the odds ratio were most often ≤ 2 .³ Animal models have demonstrated that some actors of the haemostatic system may participate in normal implantation and placental development regardless of the

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3 coagulation process.^{4,5} In humans, a basal prothrombotic state outside of pregnancy has been
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5 measured in women with previous RM and without known thrombophilia.⁶⁻⁸ This relative
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7 prothrombotic state, measured at distance of any obstetrical event, could reflect chronic
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9 endothelium damage in those women.^{9,10} Notwithstanding, the clinical trials that have assessed
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11 antithrombotic treatments (aspirin initiated before or after conception, eventually combined
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13 with LMWH, or LMWH alone initiated after pregnancy diagnosis) did not show any benefit
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15 in prevention of further loss.¹¹⁻¹⁴ Likewise, no benefit of LMWH has been shown in the
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17 subgroup of women with inherited thrombophilia (TIPPS study).¹⁵ However, we emphasize
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19 that the subcutaneous route of LMWH administration does not allow assessing this treatment
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21 at the critical time of implantation onset in fertile women. Indeed, the injections cannot be
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23 routinely initiated before 5 weeks' gestation.
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27 Regarding immune dysfunction, apart from the detection of many auto-antibodies
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29 (antiphospholipid, anti-thyroid),¹⁶ attention is now focused on natural killer cells, whose
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31 temporal and spatial distribution in the uterine mucosa suggests that they contribute to control
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33 trophoblast invasion and cytokine response at the maternal-fetal interface (balances Th-1 / Th-
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35 2 and Th-17 / T-reg). Th-2 and T-reg preponderance in normal pregnancy shifts to Th-1 and
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37 Th-17 predominance in RM.^{17,18} Otherwise, an overexpression of TOLL receptors in decidua
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39 was observed in women with RM, especially TLR4 and TLR9 receptors.¹⁹
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41 Immunomodulatory treatments have therefore been proposed and assessed without conclusive
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43 results (paternal leukocyte immunization,²⁰ intravenous immunoglobulins,²¹ Small doses of
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45 corticosteroids.)²²
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49 Despite numerous fundamental research publications and clinical trials, the only current
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51 recommendation for follow-up of child bearing women suffering from unexplained RM relies
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53 on cocooning.^{17,23}
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RM in the presence of antiphospholipid (APL) antibodies

Antiphospholipid syndrome (APS) is diagnosed in the presence of persistent, above the 99th percentile, APL levels and of at least one clinical manifestation (obstetrical, arterial or venous thrombosis). «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 »²⁴ delineates one of the possible manifestation of APS. Even though RM is one accepted clinical manifestation of APS, its specificity is low, as compared to the occurrence of fetal death after 10 weeks of amenorrhea.²⁴ The benefit of low dose aspirin and/or LMWH or heparins for secondary prevention of RM has not been demonstrated in women who present with APL antibodies and no other clinical manifestation of APS.²⁵ The HEPASA study found no benefit of combination therapy of LMWH and low dose aspirin in women who presented with APL, without arterial or venous thrombosis history, and who had at least two fetal losses before 32 weeks of amenorrhea.²⁵ As a consequence, no clinical evidence has been drawn for an optimal management of these women.

Rationale for hydroxychloroquine (HCQ)

Chloroquine and HCQ are used for centuries to treat malaria. Because of a more favorable usage profile and immunomodulatory properties, HCQ use became common for treatment of autoimmune and inflammatory diseases. Other therapeutic properties of HCQ have since been discovered, or rather rediscovered, and assessed in a more consistent manner. The following HCQ properties could be effective against mechanisms of RM.

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3 Since 30 years, benefit of HCQ to prevent venous thromboembolism (VTE) has been
4 suspected²⁶⁻³⁵ i) among patients with SLE^{26,31} and ii) among all-comer patients, free of
5 autoimmune disease, after orthopedic surgery.²⁷⁻³⁰ Thus, before the use of LMWH, HCQ was
6 assessed in the 1970s in the prevention of VTE events after orthopedic surgery. The results of
7 randomized trials,²⁸⁻³⁰ (around 10 000 patients included) led to the conclusion that HCQ was
8 beneficial in preventing VTE after orthopedic surgery.²⁸ In patients with SLE, in the presence
9 or absence of APL antibodies, studies have suggested the benefit of HCQ for the primary
10 prevention of both arterial and venous thrombotic events.^{26,31-33} In primary APS, HCQ
11 reduced the thrombus size after vessel injury in mice³⁴ and would improve the secondary
12 prevention of VTE in humans, in comparison to oral anticoagulants used alone.³⁵ The
13 antithrombotic action of HCQ would be inherent to some biological effects (in the absence³⁶⁻
14 ³⁸or in the presence³⁹⁻⁴⁰ of APL antibodies). Otherwise, much data support an endothelium
15 protective action of HCQ via anti-diabetic,⁴¹ lipidlowering,⁴² anti-oxidant⁴³ effects or a direct
16 endothelial protection, via ERK5 protein kinase activation.⁴⁴

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18 Regarding immunomodulation, HCQ has an impact on the innate immunity by
19 inhibition of the activation of some ToLL receptors (3,7,9).⁴⁵⁻⁴⁷ HCQ decreases the circulating
20 levels of interleukine 1, 2,⁴⁸ 6,⁴⁹ TNF^{49,50} and, interferon- γ ,⁵¹ promoting the TH2 processes
21 that prevail in a “normal pregnancy». Otherwise, HCQ decreases APL plasma levels⁵² and
22 interferes with both endothelial cell activation and TNF- α production, 2 major key pathways
23 involved in APS.⁵³⁻⁵⁵

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25 We also outline the well-known anti-infectious action of HCQ which originates from
26 the alkalization of intracellular acidic vesicles and might inhibit the growth of intracellular
27 microorganisms. This could act against chronic endometritis, additional mechanism suspected
28 in RM.

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3 Remarkably, the use of HCQ (and closely related molecules) as anti-malarial or in auto-
4 immune diseases during pregnancy^{56,58} and lactation⁵⁷ have provided extensive safety data
5 during pregnancy and even during breast-feeding.
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10 Otherwise, oral administration of HCQ facilitates its prescription early, before conception,
11 thus enabling fetal exposure from the very beginning of time period at risk for activation of
12 RM underlying mechanisms. Noteworthy, HCQ has never been evaluated in the indication of
13 RM with or without APL antibodies.
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19 Taken all together and given that RM is a stereotyped clinical entity whatever the maternal
20 thrombophilic status, we aimed at evaluating the role of HCQ in prevention of RM, with or
21 without APL antibodies or inherited thrombophilia.
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STUDY OBJECTIVES

Primary objective

The aim of this placebo-controlled, double-blind trial is to investigate whether HCQ (orally administered before conception until 10 weeks of gestational age) would improve the live-birth rate of 15% or more, in women with previous RM, irrespective of their biological thrombophilic status.

Secondary objectives

We aim to investigate whether:

- HCQ effect would be different among women with inherited thrombophilia or APL antibodies
- HCQ would have an impact on the occurrence of some pregnancy events (early miscarriage, intrauterine fetal death, placental vascular disease)
- The impact of HCQ would be different in subgroups of women at higher or lower risk of recurrence according to the number of previous miscarriages (= 3 ou > 3), the maternal age (≥ 35 ou < 35 ans), the parity: previous live-birth, a previous late fetal death after 10 weeks' gestation.

We aim to confirm that HCQ has no negative impact on the chance of getting pregnant, the newborn (gestational age, birth weight, survival at 28 days, congenital abnormalities), the child at 6 months.

METHODS

Study design

This is a prospective, randomized 1:1 placebo-controlled, double-blind, and French multi-centre trial of phase III, with 2 parallel-groups (HCQ 400 mg or placebo). At randomization, the minimization method will be used to balance between the 2 groups and 2 main determinants of miscarriage recurrence: maternal age (≤ 35 or >35 years) and number of previous losses (3 or ≥ 4).

Population

The study population consists of women trying to conceive and who had experienced at least 3 consecutive miscarriages at the first trimester of pregnancy (normal parental karyotypes, no uterine cavity abnormality that might explain the losses). Those women are followed-up by their gynecologist or general practitioner who refers them to one of the RM specialized unit participating in the study for screening. At the inclusion visit, the medical investigator checks inclusion and exclusion criteria of eligible women.

Inclusion criteria

- Women aged between 18 and 37 inclusive,
- Women trying to conceive,
- Women with at least 3 previous consecutive miscarriages in the first pregnancy trimester, of unknown origin defined as:
 - Normal parental karyotypes,
 - No uterine cavity abnormality that might explain RM (ultrasound scan, hysteroscopy or hystero-graphy),

- In case of persistent positive APL antibodies according to the biological criteria of Myakis:²⁴ no previous thrombotic or obstetrical event defined in APS²⁴, except for RM in the first trimester of pregnancy, .
- Women who have given their informed consent.

Exclusion criteria

- Ongoing pregnancy,
- Normal pregnancy (live and viable birth) since the last miscarriage,
- Abnormal parental karyotype,
- Uterine cavity abnormality that might explain RM in the first trimester of pregnancy,
- Antiphospholipid Syndrome defined as both:
 - persistent positive APL antibodies: lupus anticoagulant and/or APL (anticardiolipin or anti β 2 GPI, IgG or IgM) titers > 99th percentile or >40 with at least 12 weeks interval between two positive determinations (persistent antibodies) AND,
 - a specific clinical setting of APS (thrombotic or obstetrical, apart from RM in the first trimester of pregnancy) according to Myakis criteria.²⁴
- Known contraindication to a treatment by HCQ (retinopathy, hypersensitivity to chloroquine or HCQ, G6PD deficiency, acute intermittent porphyria, chronic liver or kidney insufficiency, extensive cutaneous psoriasis not controlled by local treatment, significant chronic digestive or hematologic disease) or known rare disorder of lactose metabolism (excipient),
- Past history of epilepsy or psychotic disorders,
- Indication to a treatment by HCQ according to the MA (rheumatoid arthritis, Lupus, solar eczema),

- Previous exposure >4 years to chloroquine or HCQ,
- Previous inclusion in this study,
- Woman unable to consent, protected under the terms of the law, or woman deprived of liberty by judicial or administrative decision,
- non affiliation to the social security system,
- Impossible follow-up.

Allocation, randomization and blinding

After inclusion and obtaining written consent, an identification number is assigned to the patient through a secure server providing access to electronic case report forms generated by the CIC Brest (via the "Capture System" software). The randomization is done using the "Capture System" software with implementation of a randomization with minimization to balance between the 2 treatment groups according to the main determinants of miscarriage recurrence: age (≤ 35 or >35 years) and number of miscarriages (3 or ≥ 4).

The patient, the investigator and all medical and paramedic professionals taking care of the patient will be blinded to the treatment group. The packaging of placebo and HCQ will be provided in the form of capsules with identical external shape and packaging. Information on the correspondence between treatment group and the patient number will be held by both the central pharmacy of Brest CHRU and the Data Management Unit.

Intervention

Included patients are assigned to receive either 400 mg of oral HCQ per day, administered in 2 divided doses of 200 mg or 2 daily doses of oral placebo. Thus, treatment is initiated before

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3 conception and stopped at 10 weeks' gestation (weeks of amenorrhea) or after one year of
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5 treatment in the absence of pregnancy.
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10 ***Rationale for choosing oral 400 mg daily HCQ***

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13 It is the usual route of administration and dose for indications specified by the Marketing
14 authorization (MA). This choice of dose comes from the evaluation of the risk-benefit balance
15 in the MA indications and, over all, from the fact that data of good tolerance have been
16 compiled to this (usual) dosage in literature. Otherwise, in most cases, fetal development
17 stops before 10 weeks of amenorrhea. Treatment exposure is essential from the very
18 beginning of implantation, a developmental stage that can potentially be disturbed by the
19 mechanisms responsible for miscarriage. The oral administration allows starting the treatment
20 before conception. This drug has a long half-life (from 30 to 60 days). Its full effect is
21 obtained only after certain duration of exposure. That is why its administration is required
22 before conception and its discontinuation after 10 weeks of amenorrhea, i.e. before the end of
23 the first trimester of pregnancy which is at the highest risk for RM.
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41 ***Treatments frequently given in combination***

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Study plan and procedures

Before pregnancy, women are monthly contacted by phone. The non pregnant women are systematically seen in consultation after 6 and 12 months of treatment. In the absence of pregnancy after one year, the treatment will be withdrawn and patients will be contacted by phone at 14 months and 18 months (End-of-Study visit).

During pregnancy, women are monthly seen in consultation (4 first months) or contacted by phone. The treatment is withdrawn at 10 weeks' gestation or earlier in case of pregnancy loss.

After delivery, the women are seen in consultation at ~2 months, and contacted by phone at 6 months (End-of-Study visit).

Four tubes of 2.7 ml with sodium citrate and 4 tubes SST of 5 ml (for plasma and serum bank, respectively) are collected at both the inclusion visit and the visit of pregnancy onset or the visit planned after one year of treatment in the absence of pregnancy. Additionally 2 tubes EDTA of 5 ml are collected for DNA bank at inclusion. The biological collection of plasma is carried out within 3 hours after receipt of tubes. DNA extraction is carried out within one week of collection (and at the latest within a month if the sample is frozen at -80°C pending extraction). Those specimens are designed to identify prognostic factors (including thrombophilic work-up) for efficacy of the study treatment.

End points

The primary end point is a live and viable birth.

The secondary end points are:

- A live and viable birth, for the subgroup analyses,

- The occurrence of miscarriage, in-utero fetal death, placental vascular disease, premature delivery,
- Gestational age at miscarriage,
- Concerning the child: gestational age and weight at birth, survival at 28 days, safety data at 6 months of life, congenital abnormality.

Safety considerations, safety monitoring and AE reporting

Apart from, adverse events (AE) and serious adverse events (SAE), some safety parameters will be systematically recovered: CBC, visual and neurological symptoms for women and reports of ultrasound and post-natal medical visits for children. Adverse events (AE) are defined as any untoward medical occurrence in a person who consents to biomedical research whether this event is related or not to the research or experimental drug on which this research addresses. The gravity of serious adverse events (SAE) is defined by one of the following findings: death, life-threatening situation, significant or sustained incapacity or disability, hospitalization, prolongation of hospitalization, deformity or birth defect, potentially serious event. Any SAE or event of interest, regardless of its causal relationship with the trial treatment or the research, must be reported to the sponsor, as soon as possible and the latest 24 hrs after their occurrence for SAE. The investigator is in charge of noting and reporting all SAE occurring during the study, from the date of the signed informed consent, during the study's expected duration of follow up (6 months after the treatment cessation for women and at age 6 months for newborns). Furthermore, regardless of the event occurrence deadline after the end of the study, all SAE that may be due to the research must be declared to the sponsor when there is no other cause and effect accounted for (such as serious events that may occur long after exposure to the drug, such as cancers or congenital abnormalities).

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3 Some AE and SAE are expected and can be linked to i) pregnancy, ii) the disease (occurrence
4 of another miscarriage) or study treatment (benign, adverse reaction AR, as digestive
5 symptoms or severe, serious adverse reaction SAR as skin reaction, which requires to
6 immediately stop the treatment).
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11 12 13 14 15 ***Independent Data and safety monitoring Board (IDSMB)*** 16

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18 The IDSMB comprises 8 members without competing interests, not directly involved in other
19 aspects of the trial and independent from the sponsor: 7 voting members, experts in
20 complementary fields of the pathology and clinical trials (internal medicine, methodology,
21 specialist of teratogenic agents, neonatology, hematology, ophthalmology) and a non-voting
22 independent statistician. IDSMB monitors the data that affects patient safety, provides
23 independent expertise for the evaluation of adverse events (AE) occurring during the study,
24 expresses an opinion concerning the benefit / risk ratio and provides recommendations in
25 order to help the steering committee to take decisions on protocol modification or early
26 termination of the study. The members of the IDSMB will have to meet every 20 inclusions.
27 The IDSMB will transmit its recommendations to the steering Committee, which decides
28 whether or not to stop the study. Given the safety data and the treatment benefit, several
29 different recommendations can be provided by the IDSMB: i) continuation of study without
30 protocol modification, ii) continuation of study with modification of protocol, iii) temporarily
31 discontinuation of inclusions, iiiii) early termination of the study
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49 Over the course of study, the IDSMB will have an advisory role in reassessing the benefit/risk
50 ratio of the study.
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Number of patients

The total number of scheduled patients to be recruited is 300, on the basis of the following hypotheses:

- 80% of the included women should get pregnant (ALIFE study).¹¹
- Among women who will get pregnant, we suppose that the rate of achieved pregnancy will be of 70% in the placebo group (PREFIX study).¹⁴

To demonstrate a minimal clinically important difference of 15% in live-birth rates on HCQ (i.e. a 50% relative risk reduction in pregnancy loss), we need to enroll 300 women for a power of 80%, and a two-tailed alpha risk of 5%.

The anticipated duration of recruitment is 3 years and the duration of participation of each patient: from 7 to 29 months.

Analyses plan

Analyses will be firstly conducted on an intention to treat basis for all women who have started pregnancy. Hierarchical analysis including three steps is planned :comparison of the number of live and viable births, between the two treatment groups i) among all the women, ii) among women without known APL antibodies, iii) among women without known thrombophilia (APL or inherited thrombophilia). We plan to adjust for age and number of previous losses if the difference between the two treatment groups is statistically significant ($p < .05$).

Secondary analyses will be conducted on an intention to treat mode:

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3 For all included women, comparison of the number of live and viable births between the two
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5 treatment groups.
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8 Among women who will get pregnant:
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- 10 ➤ Measure of treatment effect in women at higher risk of further miscarriage,
11 according to the presence of thrombophilia, past history of intrauterine fetal
12 death after 10 week's gestation, past history of miscarriage, > 3 previous
13 miscarriage or, no previous newborn baby,
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- 15 ➤ Comparison between the two treatment groups of the occurrence of several
16 obstetrical events (miscarriage and intrauterine fetal death, vasculo placental
17 disease, premature birth),
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- 19 ➤ Comparison between the two treatment groups of newborn clinical data
20 (gestation time at delivery, birth weight adjusted to the gestation time, number
21 of newborn babies living at 28 days and congenital abnormalities).
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33 A "per-protocol analysis" (primary endpoint) will include only patients properly exposed to
34 the study treatment, that is i) at least during a whole menstrual cycle before pregnancy onset
35 and until 10 weeks of amenorrhea ii) compliance \geq 80%.
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40 No unblinded interim analysis is planned.
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43 The biological collection will be carried out by the CRB of the CHRU of BREST and
44 integrated into the collection "thrombosis" declared to the Ministry (2008-214/ DC-2009-
45 925).
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53 **DISCUSSION**

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3 To date, there has not yet been an optimal therapy with conclusive clinical benefit for
4 unexplained RM, in the absence or presence of inherited thrombophilia. Providing some
5 psychological support seems to be the only available therapy for women with unexplained
6 RM. Although the probability of a further normal pregnancy seems high, (~70% at age 32
7 after 3 consecutive miscarriages),⁵⁹ the proposed therapeutic interventions are sometimes
8 excessive, (e.g. possible side effects) and costly (e.g. intravenous immunoglobulins, assisted
9 procreation). Therefore, it is of utmost importance to investigate other therapeutic options.
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18 In RM women with high titers of APL antibodies but without any other previous clinical
19 event listed in APS (thrombotic or obstetric event), benefit of antithrombotic treatment
20 (aspirin or LMWH) remains controversial. Clinical benefit of HCQ has not been demonstrated
21 in prospective studies with robust methodology.⁶⁰
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28 In conclusion, the benefit/risk ratio and low cost are strong incentives for assessment of HCQ
29 in unexplained RM irrespective of maternal thrombophilic status.
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36 **SPONSORSHIP**

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39 This study was sponsored by Brest University Hospital.
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46 **PATIENT AND PUBLIC INVOLVEMENT**

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49 Patients were not involved in the design of the study.
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55 **ETHICS, REGULATORY APPROVALS AND DISSEMINATION**

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3 Agreement from the French National Public Health and Drug Security Agency (160765A-22)
4 and ethical approval from the Committee for the Protection of Persons of NORD-OUEST I ()
5 have been obtained on November 4th 2016 and March 2nd 2017, respectively.
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10 This trial is registered at www.ClinicalTrials.gov as # NCT0316513. and the French National
11 Health and Drug Safety Agency (EudraCT # 2016-001330-97).
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44 **Contributors**

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46 EP conceived and designed the study. EP drafted the original grant proposal and trial
47 protocol. LDSM provides methodological and statistical expertise. GM has assisted in
48 developing the protocol, helped with implementation and has responsibilities for day-to-day
49 running of the trial including participant recruitment, data collection and liaising with other
50 sites. JH and PM have helped with protocol implementation. CB, CC, FB, VLS, GPB, DM
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3 have participated in the design of the study. VC coordinates treatment production and
4 dispensation. All authors critically reviewed and approved the final version of the manuscript.
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24 **Patient consent** Obtained.
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Hydroxychloroquine for Prevention of Recurrent Miscarriage: Study Protocol for a Multicenter Randomized Placebo Controlled Trial

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Hydroxychloroquine for Prevention of Recurrent Miscarriage:

Study Protocol for a Multicenter Randomized Placebo Controlled Trial

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ABSTRACT

Introduction Recurrent miscarriage (RM), defined by ≥ 3 consecutive losses during the first trimester of pregnancy, affects 1 to 2% of fertile couples. Standard investigations fail to reveal any apparent cause in ~ 50% of couples. However, on the basis of animal models and clinical studies, several hypotheses have been put forward concerning underlying mechanisms of RM: altered ovarian reserve, progesterone defect, thrombotic and/or endothelial dysfunction, immunological disturbances. Nonetheless, no study has yet reached conclusive beneficial clinical evidence for a potential treatment in unexplained RM. Hydroxychloroquine (HCQ) is a molecule with extensive safety data during pregnancy. HCQ pharmacological properties (e.g. anti-thrombotic, vascular protective, immunomodulatory, improved glucose tolerance, lipidlowering, antiinfectious) could be effective against some mechanisms of unexplained RM. Furthermore, eventhough clinical benefit of HCQ is suggested in prevention of thrombotic and late obstetric events in antiphospholipid (APL) syndrome, there is no data suggesting the benefit of HCQ in RM in the presence of APL antibodies.

Methods and analysis Taken all together and given the low cost of HCQ, the aim of this multicenter, randomized placebo-controlled, double-blind study is to investigate whether HCQ would improve the live-birth rate in women with RM, irrespective of maternal thrombophilic status: i) no known thrombophilia, ii) inherited thrombophilia or iii) APL antibodies. The primary end-point is a live and viable birth. After confirming eligibility and obtaining consent, 300 non-pregnant women will be randomized in 2 parallel groups for a daily oral treatment (HCQ 400 mg or placebo), initiated before conception and stopped at 10 weeks' gestation. If pregnancy doesn't occur after one year, the treatment will be stopped.

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3 **Ethics and dissemination** Agreement from the French National Public Health and Drug
4 Security Agency (160765A-22) and ethical approval from the Committee for the Protection of
5 Persons of NORD-OUEST I (2016-001330-97) have been obtained.
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10 **Trial registration number** ClinicalTrials.gov: NCT0316513
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STRENGTHS AND LIMITATIONS OF THIS STUDY

- Despite numerous fundamental research publications and clinical trials, the only recommendation that applies to follow-up of child bearing women suffering from RM relies on cocooning.
- This is the first randomized placebo-controlled study which aims to investigate whether oral hydroxychloroquine would improve the live-birth rate in women with recurrent miscarriage irrespective of maternal thrombophilic status.
- As a possible limitation, this study is based on the hypothesis that recurrent miscarriage often results from combined failures which could benefit from the pleiotropic effects of hydroxychloroquine.
- As hydroxychloroquine should probably be administered at least 2 menstrual cycles before conception to have an effect, women will be advised not to get pregnant during this period, but we do not plan to exclude those who will conceive too early.

INTRODUCTION

Background

RM is a common public health reproductive burden causing both physical and emotional distress. To date there is no treatment whose benefit has been clearly proved, even in the presence of well-known risk factors of RM. Most importantly, the absence of benefit has been clearly demonstrated for some treatments that are yet empirically proposed.

Unexplained Recurrent Miscarriage (RM)

A sporadic miscarriage is clinically detected in approximately 10% to 15% of pregnancies. Fetal development usually stops before 10 weeks.¹ Given the recurrent miscarriage (RM) frequency among fertile couples (1% to 2%) being significantly higher than the expected random one (153 = 0.34 %), RM is most often defined as ≥ 3 consecutive losses. Apart from the detection of a lethal chromosomal abnormality on products of conception, the underlying mechanism of loss remains unknown in most cases. The rate of normal embryonic karyotypes in RM steadily increases from the third loss, suggesting alternative mechanisms than meiotic aberrations.² Standard investigations fail to reveal any apparent cause in ~50% of the women. However, On the basis of animal models and clinical studies, several hypotheses have been put forward. Here, we focus on thrombosis and both endothelial and immune dysfunctions. Those could be targeted by pharmacological properties of hydroxychloroquine (HCQ).

An association with some inherited thrombophilia has been reported (factor V Leiden, mutation G20210A of the prothrombin and protein S deficiency), although the odds ratio were most often ≤ 2 .³ Animal models have demonstrated that some actors of the haemostatic system may participate in normal implantation and placental development regardless of the

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3 coagulation process.^{4,5} In humans, a basal prothrombotic state outside of pregnancy has been
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5 measured in women with previous RM and without known thrombophilia.⁶⁻⁸This relative
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7 prothrombotic state, measured at distance of any obstetrical event, could reflect chronic
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9 endothelium damage in those women.^{9,10}Notwithstanding, the clinical trials that have assessed
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11 antithrombotic treatments (aspirin initiated before or after conception, eventually combined
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13 with LMWH, or LMWH alone initiated after pregnancy diagnosis) did not show any benefit
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15 in prevention of further loss.¹¹⁻¹⁴Likewise, no benefit of LMWH has been shown in the
16
17 subgroup of women with inherited thrombophilia (TIPPS study).¹⁵However, we emphasize
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19 that the subcutaneous route of LMWH administration does not allow assessing this treatment
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21 at the critical time of implantation onset in fertile women. Indeed, the injections cannot be
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23 routinely initiated before 5 weeks' gestation.
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27 Regarding immune dysfunction, apart from the detection of many auto-antibodies
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29 (antiphospholipid, anti-thyroid),¹⁶ attention is now focused on natural killer cells, whose
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31 temporal and spatial distribution in the uterine mucosa suggests that they contribute to control
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33 trophoblast invasion and cytokine response at the maternal-fetal interface (balances Th-1 / Th-
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35 2 and Th-17 / T-reg). Th-2 and T-reg preponderance in normal pregnancy shifts to Th-1 and
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37 Th-17 predominance in RM.^{17,18} Otherwise, an overexpression of TOLL receptors in decidua
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39 was observed in women with RM, especially TLR4 and TLR9 receptors.¹⁹
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41 Immunomodulatory treatments have therefore been proposed and assessed without conclusive
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43 results (paternal leukocyte immunization,²⁰ intravenous immunoglobulins,²¹ Small doses of
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45 corticosteroids.)²²
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49 Despite numerous fundamental research publications and clinical trials, the only current
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51 recommendation for follow-up of child bearing women suffering from unexplained RM relies
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53 on cocooning.^{17,23}
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RM in the presence of antiphospholipid (APL) antibodies

Antiphospholipid syndrome (APS) is diagnosed in the presence of persistent, above the 99th percentile, APL levels and of at least one clinical manifestation (obstetrical, arterial or venous thrombosis). «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 »²⁴ delineates one of the possible manifestation of APS. Even though RM is one accepted clinical manifestation of APS, its specificity is low, as compared to the occurrence of fetal death after 10 weeks of amenorrhea.²⁴ The benefit of low dose aspirin and/or LMWH or heparins for secondary prevention of RM has not been demonstrated in women who present with APL antibodies and no other clinical manifestation of APS.²⁵ The HEPASA study found no benefit of combination therapy of LMWH and low dose aspirin in women who presented with APL, without arterial or venous thrombosis history, and who had at least two fetal losses before 32 weeks of amenorrhea.²⁵ As a consequence, no clinical evidence has been drawn for an optimal management of these women.

Rationale for hydroxychloroquine (HCQ)

Chloroquine and HCQ are used for centuries to treat malaria. Because of a more favorable usage profile and immunomodulatory properties, HCQ use became common for treatment of autoimmune and inflammatory diseases. Other therapeutic properties of HCQ have since been discovered, or rather rediscovered, and assessed in a more consistent manner. The following HCQ properties could be effective against mechanisms of RM.

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3 Since 30 years, benefit of HCQ to prevent venous thromboembolism (VTE) has been
4 suspected²⁶⁻³⁵ i) among patients with SLE^{26,31} and ii) among all-comer patients, free of
5 autoimmune disease, after orthopedic surgery.²⁷⁻³⁰ Thus, before the use of LMWH, HCQ was
6 assessed in the 1970s in the prevention of VTE events after orthopedic surgery. The results of
7 randomized trials,²⁸⁻³⁰ (around 10 000 patients included) led to the conclusion that HCQ was
8 beneficial in preventing VTE after orthopedic surgery.²⁸ In patients with SLE, in the presence
9 or absence of APL antibodies, studies have suggested the benefit of HCQ for the primary
10 prevention of both arterial and venous thrombotic events.^{26,31-33} In primary APS, HCQ
11 reduced the thrombus size after vessel injury in mice³⁴ and would improve the secondary
12 prevention of VTE in humans, in comparison to oral anticoagulants used alone.³⁵ The
13 antithrombotic action of HCQ would be inherent to some biological effects (in the absence³⁶⁻
14³⁸ or in the presence³⁹⁻⁴⁰ of APL antibodies). Otherwise, much data support an endothelium
15 protective action of HCQ via anti-diabetic,⁴¹ lipidlowering,⁴² anti-oxidant⁴³ effects or a direct
16 endothelial protection, via ERK5 protein kinase activation.⁴⁴

17
18 Regarding immunomodulation, HCQ has an impact on the innate immunity by
19 inhibition of the activation of some ToLL receptors (3,7,9).⁴⁵⁻⁴⁷ HCQ decreases the circulating
20 levels of interleukine 1, 2,⁴⁸ 6,⁴⁹ TNF^{49,50} and, interferon- γ ,⁵¹ promoting the TH2 processes
21 that prevail in a "normal pregnancy". Otherwise, HCQ decreases APL plasma levels⁵² and
22 interferes with both endothelial cell activation and TNF- α production, 2 major key pathways
23 involved in APS.⁵³⁻⁵⁵

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25 We also outline the well-known anti-infectious action of HCQ which originates from
26 the alkalization of intracellular acidic vesicles and might inhibit the growth of intracellular
27 microorganisms. This could act against chronic endometritis, additional mechanism suspected
28 in RM.

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3 Remarkably, the use of HCQ (and closely related molecules) as anti-malarial or in auto-
4 immune diseases during pregnancy^{56,58} and lactation⁵⁷ have provided extensive safety data
5 during pregnancy and even during breast-feeding.
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10 Otherwise, oral administration of HCQ facilitates its prescription early, before conception,
11 thus enabling fetal exposure from the very beginning of time period at risk for activation of
12 RM underlying mechanisms. Noteworthy, HCQ has never been evaluated in the indication of
13 RM with or without APL antibodies.
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19 Taken all together and given that RM is a stereotyped clinical entity whatever the maternal
20 thrombophilic status, we aimed at evaluating the role of HCQ in prevention of RM, with or
21 without APL antibodies or inherited thrombophilia.
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STUDY OBJECTIVES

Primary objective

The aim of this placebo-controlled, double-blind trial is to investigate whether HCQ (orally administered before conception until 10 weeks of gestational age) would improve the live-birth rate of 15% or more, in women with previous RM, irrespective of their biological thrombophilic status.

Secondary objectives

We aim to investigate whether:

- HCQ effect would be different among women with inherited thrombophilia or APL antibodies
- HCQ would have an impact on the occurrence of some pregnancy events (early miscarriage, intrauterine fetal death, placental vascular disease)
- The impact of HCQ would be different in subgroups of women at higher or lower risk of recurrence according to the number of previous miscarriages (= 3 ou > 3), the maternal age (≥ 35 ou < 35 ans), the parity: previous live-birth, a previous late fetal death after 10 weeks' gestation.

We aim to confirm that HCQ has no negative impact on the chance of getting pregnant, the newborn (gestational age, birth weight, survival at 28 days, congenital abnormalities), the child at 6 months.

METHODS

Study design

This is a prospective, randomized 1:1 placebo-controlled, double-blind, and French multi-centre trial of phase III, with 2 parallel-groups (HCQ 400 mg or placebo). At randomization, the minimization method will be used to balance between the 2 groups and 2 main determinants of miscarriage recurrence: maternal age (≤ 35 or >35 years) and number of previous losses (3 or ≥ 4).

Population

The study population consists of women trying to conceive and who had experienced at least 3 consecutive miscarriages at the first trimester of pregnancy (normal parental karyotypes, no uterine cavity abnormality that might explain the losses). Those women are followed-up by their gynecologist or general practitioner who refers them to one of the RM specialized unit participating in the study for screening. At the inclusion visit, the medical investigator checks inclusion and exclusion criteria of eligible women.

Inclusion criteria

- Women aged between 18 and 37 inclusive,
- Women trying to conceive,
- Women with at least 3 previous consecutive miscarriages in the first pregnancy trimester, of unknown origin defined as:
 - Normal parental karyotypes,
 - No uterine cavity abnormality that might explain RM (ultrasound scan, hysteroscopy or hystero-graphy),

- In case of persistent positive APL antibodies according to the biological criteria of Myakis:²⁴ no previous thrombotic or obstetrical event defined in APS²⁴, except for RM in the first trimester of pregnancy, .
- Women who have given their informed consent.

Exclusion criteria

- Ongoing pregnancy,
- Normal pregnancy (live and viable birth) since the last miscarriage,
- Abnormal parental karyotype,
- Uterine cavity abnormality that might explain RM in the first trimester of pregnancy,
- Antiphospholipid Syndrome defined as both:
 - persistent positive APL antibodies: lupus anticoagulant and/or APL (anticardiolipin or anti β 2 GPI, IgG or IgM) titers > 99th percentile or >40 with at least 12 weeks interval between two positive determinations (persistent antibodies) AND,
 - a specific clinical setting of APS (thrombotic or obstetrical, apart from RM in the first trimester of pregnancy) according to Myakis criteria.²⁴
- Known contraindication to a treatment by HCQ (retinopathy, hypersensitivity to chloroquine or HCQ, G6PD deficiency, acute intermittent porphyria, chronic liver or kidney insufficiency, extensive cutaneous psoriasis not controlled by local treatment, significant chronic digestive or hematologic disease) or known rare disorder of lactose metabolism (excipient),
- Past history of epilepsy or psychotic disorders,
- Indication to a treatment by HCQ according to the MA (rheumatoid arthritis, Lupus, solar eczema),

- Previous exposure >4 years to chloroquine or HCQ,
- Previous inclusion in this study,
- Woman unable to consent, protected under the terms of the law, or woman deprived of liberty by judicial or administrative decision,
- non affiliation to the social security system,
- Impossible follow-up.

Allocation, randomization and blinding

After inclusion and obtaining written consent, an identification number is assigned to the patient through a secure server providing access to electronic case report forms generated by the CIC Brest (via the "Capture System" software). The randomization is done using the "Capture System" software with implementation of a randomization with minimization to balance between the 2 treatment groups according to the main determinants of miscarriage recurrence: age (≤ 35 or >35 years) and number of miscarriages (3 or ≥ 4).

The patient, the investigator and all medical and paramedic professionals taking care of the patient will be blinded to the treatment group. The packaging of placebo and HCQ will be provided in the form of capsules with identical external shape and packaging. Information on the correspondence between treatment group and the patient number will be held by both the central pharmacy of Brest CHRU and the Data Management Unit.

Intervention

Included patients are assigned to receive either 400 mg of oral HCQ per day, administered in 2 divided doses of 200 mg or 2 daily doses of oral placebo. Thus, treatment is initiated before

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3 conception and stopped at 10 weeks' gestation (weeks of amenorrhea) or after one year of
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5 treatment in the absence of pregnancy.
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10 ***Rationale for choosing oral 400 mg daily HCQ***

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13 It is the usual route of administration and dose for indications specified by the Marketing
14 authorization (MA). This choice of dose comes from the evaluation of the risk-benefit balance
15 in the MA indications and, over all, from the fact that data of good tolerance have been
16 compiled to this (usual) dosage in literature. Otherwise, in most cases, fetal development
17 stops before 10 weeks of amenorrhea. Treatment exposure is essential from the very
18 beginning of implantation, a developmental stage that can potentially be disturbed by the
19 mechanisms responsible for miscarriage. The oral administration allows starting the treatment
20 before conception. This drug has a long half-life (from 30 to 60 days). Its full effect is
21 obtained only after certain duration of exposure. That is why its administration is required
22 before conception and its discontinuation after 10 weeks of amenorrhea, i.e. before the end of
23 the first trimester of pregnancy which is at the highest risk for RM.
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41 ***Treatments frequently given in combination***

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44 ➤ Folic acid and other vitamin supplements,
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46 ➤ Aspirin at low dose, which could be given by some investigators to prevent the
47 recurrence of vasculo-placental disease (preeclampsia, intra-utero growth restriction)
48 or as part of a primary prevention of vasculo-placental events in women with high
49 levels of persistent APL antibodies.
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Study plan and procedures

Before pregnancy, women are monthly contacted by phone. The non pregnant women are systematically seen in consultation after 6 and 12 months of treatment. In the absence of pregnancy after one year, the treatment will be withdrawn and patients will be contacted by phone at 14 months and 18 months (End-of-Study visit).

During pregnancy, women are monthly seen in consultation (4 first months) or contacted by phone. The treatment is withdrawn at 10 weeks' gestation or earlier in case of pregnancy loss.

After delivery, the women are seen in consultation at ~2 months, and contacted by phone at 6 months (End-of-Study visit).

Four tubes of 2.7 ml with sodium citrate and 4 tubes SST of 5 ml (for plasma and serum bank, respectively) are collected at both the inclusion visit and the visit of pregnancy onset or the visit planned after one year of treatment in the absence of pregnancy. Additionally 2 tubes EDTA of 5 ml are collected for DNA bank at inclusion. The biological collection of plasma is carried out within 3 hours after receipt of tubes. DNA extraction is carried out within one week of collection (and at the latest within a month if the sample is frozen at -80°C pending extraction). Those specimens are designed to identify prognostic factors (including thrombophilic work-up) for efficacy of the study treatment.

End points

The primary end point is a live and viable birth.

The secondary end points are:

- A live and viable birth, for the subgroup analyses,

- The occurrence of miscarriage, in-utero fetal death, placental vascular disease, premature delivery,
- Gestational age at miscarriage,
- Concerning the child: gestational age and weight at birth, survival at 28 days, safety data at 6 months of life, congenital abnormality.

Safety considerations, safety monitoring and AE reporting

Apart from, adverse events (AE) and serious adverse events (SAE), some safety parameters will be systematically recovered: CBC, visual and neurological symptoms for women and reports of ultrasound and post-natal medical visits for children. Adverse events (AE) are defined as any untoward medical occurrence in a person who consents to biomedical research whether this event is related or not to the research or experimental drug on which this research addresses. The gravity of serious adverse events (SAE) is defined by one of the following findings: death, life-threatening situation, significant or sustained incapacity or disability, hospitalization, prolongation of hospitalization, deformity or birth defect, potentially serious event. Any SAE or event of interest, regardless of its causal relationship with the trial treatment or the research, must be reported to the sponsor, as soon as possible and the latest 24 hrs after their occurrence for SAE. The investigator is in charge of noting and reporting all SAE occurring during the study, from the date of the signed informed consent, during the study's expected duration of follow up (6 months after the treatment cessation for women and at age 6 months for newborns). Furthermore, regardless of the event occurrence deadline after the end of the study, all SAE that may be due to the research must be declared to the sponsor when there is no other cause and effect accounted for (such as serious events that may occur long after exposure to the drug, such as cancers or congenital abnormalities).

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3 Some AE and SAE are expected and can be linked to i) pregnancy, ii) the disease (occurrence
4 of another miscarriage) or study treatment (benign, adverse reaction AR, as digestive
5 symptoms or severe, serious adverse reaction SAR as skin reaction, which requires to
6 immediately stop the treatment).
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11 12 13 14 15 ***Independent Data and safety monitoring Board (IDSMB)*** 16

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18 The IDSMB comprises 8 members without competing interests, not directly involved in other
19 aspects of the trial and independent from the sponsor: 7 voting members, experts in
20 complementary fields of the pathology and clinical trials (internal medicine, methodology,
21 specialist of teratogenic agents, neonatology, hematology, ophthalmology) and a non-voting
22 independent statistician. IDSMB monitors the data that affects patient safety, provides
23 independent expertise for the evaluation of adverse events (AE) occurring during the study,
24 expresses an opinion concerning the benefit / risk ratio and provides recommendations in
25 order to help the steering committee to take decisions on protocol modification or early
26 termination of the study. The members of the IDSMB will have to meet every 20 inclusions.
27 The IDSMB will transmit its recommendations to the steering Committee, which decides
28 whether or not to stop the study. Given the safety data and the treatment benefit, several
29 different recommendations can be provided by the IDSMB: i) continuation of study without
30 protocol modification, ii) continuation of study with modification of protocol, iii) temporarily
31 discontinuation of inclusions, iiiii) early termination of the study
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49 Over the course of study, the IDSMB will have an advisory role in reassessing the benefit/risk
50 ratio of the study.
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Number of patients

The total number of scheduled patients to be recruited is 300, on the basis of the following hypotheses:

- 80% of the included women should get pregnant (ALIFE study).¹¹
- Among women who will get pregnant, we suppose that the rate of achieved pregnancy will be of 70% in the placebo group (PREFIX study).¹⁴

To demonstrate a minimal clinically important difference of 15% in live-birth rates on HCQ (i.e. a 50% relative risk reduction in pregnancy loss), we need to enroll 300 women for a power of 80%, and a two-tailed alpha risk of 5%.

The anticipated duration of recruitment is 3 years and the duration of participation of each patient: from 7 to 29 months.

Analyses plan

Analyses will be firstly conducted on an intention to treat basis for all women who have started pregnancy. Hierarchical analysis including three steps is planned :comparison of the number of live and viable births, between the two treatment groups i) among all the women, ii) among women without known APL antibodies, iii) among women without known thrombophilia (APL or inherited thrombophilia). We plan to adjust for age and number of previous losses if the difference between the two treatment groups is statistically significant ($p < .05$).

Secondary analyses will be conducted on an intention to treat mode:

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3 For all included women, comparison of the number of live and viable births between the two
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5 treatment groups.
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8 Among women who will get pregnant:
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11 ➤ Measure of treatment effect in women at higher risk of further miscarriage,
12 according to the presence of thrombophilia, past history of intrauterine fetal
13 death after 10 week's gestation, past history of miscarriage, > 3 previous
14 miscarriage or, no previous newborn baby,
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19 ➤ Comparison between the two treatment groups of the occurrence of several
20 obstetrical events (miscarriage and intrauterine fetal death, vasculo placental
21 disease, premature birth),
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26 ➤ Comparison between the two treatment groups of newborn clinical data
27 (gestation time at delivery, birth weight adjusted to the gestation time, number
28 of newborn babies living at 28 days and congenital abnormalities.
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33 A "per-protocol analysis" (primary endpoint) will include only patients properly exposed to
34 the study treatment, that is i) at least during a whole menstrual cycle before pregnancy onset
35 and until 10 weeks of amenorrhea ii) compliance \geq 80%.
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40 No unblinded interim analysis is planned.
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43 The biological collection will be carried out by the CRB of the CHRU of BREST and
44 integrated into the collection "thrombosis" declared to the Ministry (2008-214/ DC-2009-
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DISCUSSION

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3 To date, there has not yet been an optimal therapy with conclusive clinical benefit for
4 unexplained RM, in the absence or presence of inherited thrombophilia. Providing some
5 psychological support seems to be the only available therapy for women with unexplained
6 RM. Although the probability of a further normal pregnancy seems high, (~70% at age 32
7 after 3 consecutive miscarriages),⁵⁹ the proposed therapeutic interventions are sometimes
8 excessive, (e.g. possible side effects) and costly (e.g. intravenous immunoglobulins, assisted
9 procreation). Therefore, it is of utmost importance to investigate other therapeutic options.
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18 In RM women with high titers of APL antibodies but without any other previous clinical
19 event listed in APS (thrombotic or obstetric event), benefit of antithrombotic treatment
20 (aspirin or LMWH) remains controversial. Clinical benefit of HCQ has not been demonstrated
21 in prospective studies with robust methodology.⁶⁰
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28 In conclusion, the benefit/risk ratio and low cost are strong incentives for assessment of HCQ
29 in unexplained RM irrespective of maternal thrombophilic status.
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36 **SPONSORSHIP**

37
38
39 This study was sponsored by Brest University Hospital.
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46 **PATIENT AND PUBLIC INVOLVEMENT**

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48
49 Patients were not involved in the design of the study.
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55 **ETHICS, REGULATORY APPROVALS AND DISSEMINATION**

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3 Agreement from the French National Public Health and Drug Security Agency (160765A-22)
4 and ethical approval from the Committee for the Protection of Persons of NORD-OUEST I ()
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6
7 have been obtained on November 4th 2016 and March 2nd 2017, respectively.
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10 This trial is registered at www.ClinicalTrials.gov as # NCT0316513. and the French National
11
12 Health and Drug Safety Agency (EudraCT # 2016-001330-97).
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44 **Contributors**

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47 EP conceived and designed the study. EP drafted the original grant proposal and trial
48
49 protocol. LDSM provides methodological and statistical expertise. GM has assisted in
50
51 developing the protocol, helped with implementation and has responsibilities for day-to-day
52
53 running of the trial including participant recruitment, data collection and liaising with other
54
55 sites. JH and PM have helped with protocol implementation. CB, CC, FB, VLS, GPB, DM
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3 have participated in the design of the study. VC coordinates treatment production and
4 dispensation. All authors critically reviewed and approved the final version of the manuscript.
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24 **Patient consent** Obtained.
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BMJ Open

**Hydroxychloroquine for Prevention of Recurrent
Miscarriage:
Study Protocol for a Multicenter Randomized Placebo
Controlled Trial
BaBy hydroxychloroQuine (BBQ) study**

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Secondary Subject Heading:	Medical management
Keywords:	Subfertility < GYNAECOLOGY, INTERNAL MEDICINE, Clinical trials < THERAPEUTICS

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Manuscripts

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3 1 Hydroxychloroquine for Prevention of Recurrent Miscarriage:
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7 2 Study Protocol for a Multicenter Randomized Placebo Controlled Trial
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10 3 **BaBy** hydroxychloroQuine (BBQ) study
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26 ABSTRACT

27 **Introduction** Recurrent miscarriage (RM), defined by ≥ 3 consecutive losses during the first
28 trimester of pregnancy, affects 1 to 2% of fertile couples. Standard investigations fail to reveal
29 any apparent cause in $\sim 50\%$ of couples. However, on the basis of animal models and clinical
30 studies, several hypotheses have been put forward concerning underlying mechanisms of RM:
31 altered ovarian reserve, progesterone defect, thrombotic and/or endothelial dysfunction,
32 immunological disturbances. Nonetheless, no study has yet reached conclusive beneficial
33 clinical evidence for a potential treatment in unexplained RM. Hydroxychloroquine (HCQ) is
34 a molecule with extensive safety data during pregnancy. HCQ pharmacological properties
35 (e.g. anti-thrombotic, vascular protective, immunomodulatory, improved glucose tolerance,
36 lipidlowering, antiinfectious) could be effective against some mechanisms of unexplained
37 RM. Furthermore, eventhough clinical benefit of HCQ is suggested in prevention of
38 thrombotic and late obstetric events in antiphospholipid (APL) syndrome, there is no data
39 suggesting the benefit of HCQ in RM in the presence of APL antibodies.

40 **Methods and analysis** Taken all together and given the low cost of HCQ, the aim of this
41 multicenter, randomized placebo-controlled, double-blind study is to investigate whether
42 HCQ would improve the live-birth rate in women with RM, irrespective of maternal
43 thrombophilic status: i) no known thrombophilia, ii) inherited thrombophilia or iii) APL
44 antibodies. The primary end-point is a live and viable birth. After confirming eligibility and
45 obtaining consent, 300 non-pregnant women will be randomized in 2 parallel groups for a
46 daily oral treatment (HCQ 400 mg or placebo), initiated before conception and stopped at 10
47 weeks' gestation. If pregnancy doesn't occur after one year, the treatment will be stopped.

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3 48 **Ethics and dissemination** Agreement from the French National Public Health and Drug
4
5 49 Security Agency (160765A-22) and ethical approval from the Committee for the Protection of
6
7 50 Persons of NORD-OUEST I (2016-001330-97) have been obtained.
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11 51 **Trial registration number** ClinicalTrials.gov: NCT0316513
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For peer review only

53 STRENGTHS AND LIMITATIONS OF THIS STUDY

- 54 ➤ Despite numerous fundamental research publications and clinical trials, the only
55 recommendation that applies to follow-up of child bearing women suffering from RM
56 relies on cocooning.
- 57 ➤ This is the first randomized placebo-controlled study which aims to investigate
58 whether oral hydroxychloroquine would improve the live-birth rate in women with
59 recurrent miscarriage irrespective of maternal thrombophilic status.
- 60 ➤ As a possible limitation, this study is based on the hypothesis that recurrent
61 miscarriage often results from combined failures which could benefit from the
62 pleiotropic effects of hydroxychloroquine.
- 63 ➤ As hydroxychloroquine should probably be administered at least 2 menstrual cycles
64 before conception to have an effect, women will be advised not to get pregnant during
65 this period, but we do not plan to exclude those who will conceive too early.

66

67 INTRODUCTION

68 **Background**

69 RM is a common public health reproductive burden causing both physical and emotional
70 distress. To date there is no treatment whose benefit has been clearly proved, even in the
71 presence of well-known risk factors of RM. Most importantly, the absence of benefit has been
72 clearly demonstrated for some treatments that are yet empirically proposed.

74 *Unexplained Recurrent Miscarriage (RM)*

75 A sporadic miscarriage is clinically detected in approximately 10% to 15% of pregnancies.
76 Fetal development usually stops before 10 weeks.¹Given the recurrent miscarriage (RM)
77 frequency among fertile couples (1% to 2%) being significantly higher than the expected
78 random one ($15 \times 15 \times 15\% = 0.34\%$), RM is most often defined as ≥ 3 consecutive losses.
79 Apart from the detection of a lethal chromosomal abnormality on products of conception, the
80 underlying mechanism of loss remains unknown in most cases. The rate of normal embryonic
81 karyotypes in RM steadily increases from the third loss, suggesting alternative mechanisms
82 than meiotic aberrations.²Standard investigations fail to reveal any apparent cause in ~50% of
83 the women. However, On the basis of animal models and clinical studies, several hypotheses
84 have been put forward. Here, we focus on thrombosis and both endothelial and immune
85 dysfunctions. Those could be targeted by pharmacological properties of hydroxychloroquine
86 (HCQ).

87 An association with some inherited thrombophilia has been reported (factor V Leiden,
88 mutation G20210A of the prothrombin and protein S deficiency), although the odds ratio were
89 most often ≤ 2 .³ Animal models have demonstrated that some actors of the haemostatic

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3 90 system may participate in normal implantation and placental development regardless of the
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5 91 coagulation process.^{4,5} In humans, a basal prothrombotic state outside of pregnancy has been
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7 92 measured in women with previous RM and without known thrombophilia.⁶⁻⁸This relative
8
9 93 prothrombotic state, measured at distance of any obstetrical event, could reflect chronic
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11 94 endothelium damage in those women.^{9,10}Notwithstanding, the clinical trials that have assessed
12
13 95 antithrombotic treatments (aspirin initiated before or after conception, eventually combined
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15 96 with LMWH, or LMWH alone initiated after pregnancy diagnosis) did not show any benefit
16
17 97 in prevention of further loss.¹¹⁻¹⁴Likewise, no benefit of LMWH has been shown in the
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19 98 subgroup of women with inherited thrombophilia (TIPPS study).¹⁵However, we emphasize
20
21 99 that the subcutaneous route of LMWH administration does not allow assessing this treatment
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23 100 at the critical time of implantation onset in fertile women. Indeed, the injections cannot be
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25 101 routinely initiated before 5 weeks' gestation.

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31 102 Regarding immune dysfunction, apart from the detection of many auto-antibodies
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33 103 (antiphospholipid, anti-thyroid),¹⁶ attention is now focused on natural killer cells, whose
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35 104 temporal and spatial distribution in the uterine mucosa suggests that they contribute to control
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37 105 trophoblast invasion and cytokine response at the maternal-fetal interface (balances Th-1 / Th-
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39 106 2 and Th-17 / T-reg). Th-2 and T-reg preponderance in normal pregnancy shifts to Th-1 and
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41 107 Th-17 predominance in RM.^{17,18} Otherwise, an overexpression of TOLL receptors in decidua
42
43 108 was observed in women with RM, especially TLR4 and TLR9 receptors.¹⁹
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45 109 Immunomodulatory treatments have therefore been proposed and assessed without conclusive
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47 110 results (paternal leukocyte immunization,²⁰ intravenous immunoglobulins,²¹ Small doses of
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49 111 corticosteroids.)²²

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55 112 Despite numerous fundamental research publications and clinical trials, the only current
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57 113 recommendation for follow-up of child bearing women suffering from unexplained RM relies
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59 114 on cocooning.^{17,23}

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56 116 ***RM in the presence of antiphospholipid (APL) antibodies***
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9 117 Antiphospholipid syndrome (APS) is diagnosed in the presence of persistent, above the 99th
10 118 percentile, APL levels and of at least one clinical manifestation (obstetrical, arterial or venous
11 119 thrombosis). «Three or more unexplained consecutive spontaneous abortions before the 10th
12 120 week of gestation, with maternal anatomic or hormonal abnormalities and paternal and
13 121 maternal chromosomal causes excluded »²⁴ delineates one of the possible manifestation of
14 122 APS. Even though RM is one accepted clinical manifestation of APS, its specificity is low, as
15 123 compared to the occurrence of fetal death after 10 weeks of amenorrhea.²⁴ The benefit of low
16 124 dose aspirin and/or LMWH or heparins for secondary prevention of RM has not been
17 125 demonstrated in women who present with APL antibodies and no other clinical manifestation
18 126 of APS.²⁵ The HEPASA study found no benefit of combination therapy of LMWH and low
19 127 dose aspirin in women who presented with APL, without arterial or venous thrombosis
20 128 history, and who had at least two fetal losses before 32 weeks of amenorrhea.²⁵ As a
21 129 consequence, no clinical evidence has been drawn for an optimal management of these
22 130 women.

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4445 132 ***Rationale for hydroxychloroquine (HCQ)***
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48 133 Chloroquine and HCQ are used for centuries to treat malaria. Because of a more favorable
49 134 usage profile and immunomodulatory properties, HCQ use became common for treatment of
50 135 autoimmune and inflammatory diseases. Other therapeutic properties of HCQ have since been
51 136 discovered, or rather rediscovered, and assessed in a more consistent manner. The following
52 137 HCQ properties could be effective against mechanisms of RM.

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3 138 Since 30 years, benefit of HCQ to prevent venous thromboembolism (VTE) has been
4
5 139 suspected²⁶⁻³⁵ i) among patients with SLE^{26,31} and ii) among all-comer patients, free of
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7 140 autoimmune disease, after orthopedic surgery.²⁷⁻³⁰ Thus, before the use of LMWH, HCQ was
8
9 141 assessed in the 1970s in the prevention of VTE events after orthopedic surgery. The results of
10
11 142 randomized trials,²⁸⁻³⁰ (around 10 000 patients included) led to the conclusion that HCQ was
12
13 143 beneficial in preventing VTE after orthopedic surgery.²⁸ In patients with SLE, in the presence
14
15 144 or absence of APL antibodies, studies have suggested the benefit of HCQ for the primary
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17 145 prevention of both arterial and venous thrombotic events.^{26,31-33} In primary APS, HCQ
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19 146 reduced the thrombus size after vessel injury in mice³⁴ and would improve the secondary
20
21 147 prevention of VTE in humans, in comparison to oral anticoagulants used alone.³⁵ The
22
23 148 antithrombotic action of HCQ would be inherent to some biological effects (in the absence³⁶⁻
24
25 149 ³⁸or in the presence³⁹⁻⁴⁰ of APL antibodies). Otherwise, much data support an endothelium
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27 150 protective action of HCQ via anti-diabetic,⁴¹ lipid lowering,⁴² anti-oxidant⁴³ effects or a direct
28
29 151 endothelial protection, via ERK5 protein kinase activation.⁴⁴

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35 152 Regarding immunomodulation, HCQ has an impact on the innate immunity by
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37 153 inhibition of the activation of some Toll receptors (3,7,9).⁴⁵⁻⁴⁷ HCQ decreases the circulating
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39 154 levels of interleukin 1, 2,⁴⁸ 6,⁴⁹ TNF^{49,50} and, interferon- γ ,⁴⁹ promoting the TH2 processes
40
41 155 that prevail in a "normal pregnancy". Otherwise, HCQ decreases APL plasma levels⁵¹ and
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43 156 interferes with both endothelial cell activation and TNF- α production, 2 major key pathways
44
45 157 involved in APS.⁵²⁻⁵⁴

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49 158 We also outline the well-known anti-infectious action of HCQ which originates from
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51 159 the alkalization of intracellular acidic vesicles and might inhibit the growth of intracellular
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53 160 microorganisms. This could act against chronic endometritis, additional mechanism suspected
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55 161 in RM.

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3 162 Remarkably, the use of HCQ (and closely related molecules) as anti-malarial or in auto-
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5 163 immune diseases during pregnancy⁵⁵⁻⁵⁷ and lactation⁵⁶ have provided extensive safety data
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7 164 during pregnancy and even during breast-feeding.
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11 165 Otherwise, oral administration of HCQ facilitates its prescription early, before conception,
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13 166 thus enabling fetal exposure from the very beginning of time period at risk for activation of
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15 167 RM underlying mechanisms. Noteworthy, HCQ has never been evaluated in the indication of
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17 168 RM with or without APL antibodies.
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21 169 Taken all together and given that RM is a stereotyped clinical entity whatever the maternal
22
23 170 thrombophilic status, we aimed at evaluating the role of HCQ in prevention of RM, with or
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25 171 without APL antibodies or inherited thrombophilia. We therefore initiated a multicenter
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27 172 placebo-controlled trial sponsored by Brest University hospital and supported by a grant from
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29 173 the French Ministry of Health (PHRCN-17-0573).
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174 **STUDY OBJECTIVES**

175 *Primary objective*

176 The aim of this placebo-controlled, double-blind trial is to investigate whether HCQ (orally
177 administrated before conception until 10 weeks of gestational age) would improve the live-
178 birth rate of 15% or more, in women with previous RM, irrespective of their biological
179 thrombophilic status.

180

181 *Secondary objectives*

182 We aim to investigate whether:

- 183 ➤ HCQ effect would be different among women with inherited thrombophilia
184 or APL antibodies
- 185 ➤ HCQ would have an impact on the occurrence of some pregnancy events
186 (early miscarriage, intrauterine fetal death, placental vascular disease)
- 187 ➤ The impact of HCQ would be different in subgroups of women at higher or
188 lower risk of recurrence according to the number of previous miscarriages
189 (= 3 ou > 3), the maternal age (≥ 35 ou < 35 ans), the parity: previous live-
190 birth, a previous late fetal death after 10 weeks' gestation.

191 We aim to confirm that HCQ has no negative impact on the chance of getting
192 pregnant, the newborn (gestational age, birth weight, survival at 28 days, congenital
193 abnormalities), the child at 6 months.

194

195 **METHODS**

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3 196 ***Study design***
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6 197 This is a prospective, randomized 1:1 placebo-controlled, double-blind, and French multi-
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8 198 centre trial of phase III (, with 2 parallel-groups (HCQ 400 mg or placebo). At randomization,
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10 199 the minimization method will be used to balance between the 2 groups and 2 main
11
12 200 determinants of miscarriage recurrence: maternal age (≤ 35 or >35 years) and number of
13
14 201 previous losses (3 or ≥ 4).
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21 203 ***Study setting***
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25 204 Women are currently being enrolled in university hospitals (gynecology units: Besançon,
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27 205 Brest, Clermont Ferrand, Lille, Marseille, Nantes, Paris Cochin, Paris Bichat, Rennes, Saint-
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29 206 Etienne; internal medicine units: Brest, Paris Saint-Antoine) or in general hospitals
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31 207 (gynecology units: Auch, Quimper, Mont de Marsan, Pau).
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38 209 ***Population***
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41 210 The study population consists of women trying to conceive and who had experienced at least
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43 211 3 consecutive miscarriages at the first trimester of pregnancy (normal parental karyotypes, no
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45 212 uterine cavity abnormality that might explain the losses). Through medical meetings, emails
46
47 213 and letters, all obstetricians and internal medicine practitioners working in each participating
48
49 214 centre's catchment area have been informed and trained on BBQ study. All of those are asked
50
51 215 to refer potentially eligible women to the unit participating in the study for screening. In each
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53 216 centre, patient recruitment is ensured by already in place settings such as specific RM
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55 217 consultations or other OBS/GYN patient management units. In addition to this recruitment
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3 218 approach, poster information in consultation waiting rooms is being used to reach-out to more
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5 219 patients.
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8 220 At the inclusion visit, the medical investigator checks inclusion and exclusion criteria of
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10 221 eligible women.
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17 223 ***Inclusion criteria***

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20 224 ➤ Women aged between 18 and 37 inclusive,

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22 225 ➤ Women trying to conceive,

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24 226 ➤ Women with at least 3 previous consecutive miscarriages in the first pregnancy
25
26 227 trimester, of unknown origin defined as:

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29 228 • Normal parental karyotypes,

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31 229 • No uterine cavity abnormality that might explain RM (ultrasound scan,
32
33 230 hysteroscopy or hystero-graphy),

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35 231 • In case of persistent positive APL antibodies according to the biological
36
37 232 criteria of Myakis:²⁴no previous thrombotic or obstetrical event defined in
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39 233 APS²⁴, except for RM in the first trimester of pregnancy, .

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41 234 ➤ Women who have given their informed consent.
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49 236 ***Exclusion criteria***

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52 237 ➤ Ongoing pregnancy,

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54 238 ➤ Normal pregnancy (live and viable birth) since the last miscarriage,

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56 239 ➤ Abnormal parental karyotype,

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58 240 ➤ Uterine cavity abnormality that might explain RM in the first trimester of pregnancy,
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3 241 ➤ Antiphospholipid Syndrome defined as both:
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5 242 • persistent positive APL antibodies: lupus anticoagulant and/or APL
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7 243 (anticardiolipin or anti béta2 GPI, IgG or IgM) titers > 99th percentile or >40
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9 244 with at least 12 weeks interval between two positive determinations (persistent
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11 245 antibodies) AND,
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14 246 • a specific clinical setting of APS (thrombotic or obstetrical, apart from RM in
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16 247 the first trimester of pregnancy) according to Myakis criteria.²⁴
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19 248 ➤ Known contraindication to a treatment by HCQ (retinopathy, hypersensitivity to
20
21 249 chloroquine or HCQ, G6PD deficiency, acute intermittent porphyria, chronic liver or
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23 250 kidney insufficiency, extensive cutaneous psoriasis not controlled by local treatment,
24
25 251 significant chronic digestive or hematologic disease) or known rare disorder of lactose
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27 252 metabolism (excipient),
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30 253 ➤ Past history of epilepsia or psychotic disorders,
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32
33 254 ➤ Indication to a treatment by HCQ according to the MA (rheumatoid arthritis, Lupus,
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35 255 solar eczema),
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37 256 ➤ Previous exposure >4 years to chloroquine or HCQ,
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40 257 ➤ Previous inclusion in this study,
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43 258 ➤ Woman unable to consent, protected under the terms of the law, or woman deprived of
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45 259 liberty by judicial or administrative decision,
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47 260 ➤ non affiliation to the social security system,
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49 261 ➤ Impossible follow-up.
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56 263 ***Allocation, randomization and blinding***
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3 264 After inclusion and obtaining written consent, an identification number is assigned to the
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5 265 patient through a secure server providing access to electronic case report forms generated by
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7 266 the CIC Brest (via the "Capture System" software). The randomization is done using the
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9 267 "Capture System" software with implementation of a randomization with minimization to
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11 268 balance between the 2 treatment groups according to the main determinants of miscarriage
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13 269 recurrence: age (≤ 35 or >35 years) and number of miscarriages (3 or ≥ 4).

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17 270 The patient, the investigator and all medical and paramedic professionals taking care of the
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19 271 patient will be blinded to the treatment group. The packaging of placebo and HCQ will be
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21 272 provided in the form of capsules with identical external shape and packaging. Information on
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23 273 the correspondence between treatment group and the patient number will be held by both the
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25 274 central pharmacy of Brest CHRU and the Data Management Unit.

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31 32 33 276 ***Intervention***

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36 277 Included patients are assigned to receive either 400 mg of oral HCQ per day, administered in
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38 278 2 divided doses of 200 mg or 2 daily doses of oral placebo. Thus, treatment is initiated before
39
40 279 conception and stopped at 10 weeks' gestation (weeks of amenorrhea) or after one year of
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42 280 treatment in the absence of pregnancy.

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47 48 49 282 ***Rationale for choosing oral 400 mg daily HCQ***

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52 283 It is the usual route of administration and dose for indications specified by the Marketing
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54 284 authorization (MA). This choice of dose comes from the evaluation of the risk-benefit balance
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56 285 in the MA indications and, over all, from the fact that data of good tolerance have been
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58 286 compiled to this (usual) dosage in literature. Otherwise, in most cases, fetal development

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3 287 stops before 10 weeks of amenorrhea. Treatment exposure is essential from the very
4
5 288 beginning of implantation, a developmental stage that can potentially be disturbed by the
6
7 289 mechanisms responsible for miscarriage. The oral administration allows starting the treatment
8
9 290 before conception. This drug has a long half-life (from 30 to 60 days). Its full effect is
10
11 291 obtained only after certain duration of exposure. That is why its administration is required
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13 292 before conception and its discontinuation after 10 weeks of amenorrhea, i.e. before the end of
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15 293 the first trimester of pregnancy which is at the highest risk for RM.
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23 295 ***Treatments frequently given in combination***

- 26 296 ➤ Folic acid and other vitamin supplements,
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28 297 ➤ Aspirin at low dose, which could be given by some investigators to prevent the
29
30 298 recurrence of vasculo-placental disease (preeclampsia, intra-utero growth restriction)
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32 299 or as part of a primary prevention of vasculo-placental events in women with high
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34 300 levels of persistent APL antibodies.
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42 302 ***Study plan and procedures***

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45 303 Before pregnancy, women are monthly contacted by phone. The non pregnant women are
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47 304 systematically seen in consultation after 6 and 12 months of treatment. In the absence of
48
49 305 pregnancy after one year, the treatment will be withdrawn and patients will be contacted by
50
51 306 phone at 14 months and 18 months (End-of-Study visit).
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55 307 During pregnancy, women are monthly seen in consultation (4 first months) or contacted by
56
57 308 phone. The treatment is withdrawn at 10 weeks' gestation or earlier in case of pregnancy loss.
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3 309 After delivery, the women are seen in consultation at ~2 months, and contacted by phone at 6
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5 310 months (End-of-Study visit).
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8 311 Four tubes of 2.7 ml with sodium citrate and 4 tubes SST of 5 ml (for plasma and serum bank,
9
10 312 respectively) are collected at both the inclusion visit and the visit of pregnancy onset or the
11
12 313 visit planned after one year of treatment in the absence of pregnancy. Additionally 2 tubes
13
14 314 EDTA of 5 ml are collected for DNA bank at inclusion. The biological collection of plasma is
15
16 315 carried out within 3 hours after receipt of tubes. DNA extraction is carried out within one
17
18 316 week of collection (and at the latest within a month if the sample is frozen at -80 ° C pending
19
20 317 extraction). Those specimens are designed to identify prognostic factors (including
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22 318 thrombophilic work-up) for efficacy of the study treatment.
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31 320 ***End points***

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34 321 The primary end point is a live and viable birth.
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37 322 The secondary end points are:
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- 40 323 ➤ A live and viable birth, for the subgroup analyses,
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42 324 ➤ The occurrence of miscarriage, in-utero fetal death, placental vascular disease,
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44 325 premature delivery,
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46 326 ➤ Gestational age at miscarriage,
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48 327 ➤ Concerning the child: gestational age and weight at birth, survival at 28 days,
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50 328 safety data at 6 months of life, congenital abnormality.
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58 330 ***Safety considerations, safety monitoring and AE reporting***

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3 331 Apart from, adverse events (AE) and serious adverse events (SAE), some safety parameters
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5 332 will be systematically recovered: CBC, visual and neurological symptoms for women and
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7 333 reports of ultrasound and post-natal medical visits for children. Adverse events (AE) are
8
9 334 defined as any untoward medical occurrence in a person who consents to biomedical research
10
11 335 whether this event is related or not to the research or experimental drug on which this research
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13 336 addresses. The gravity of serious adverse events (SAE) is defined by one of the following
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15 337 findings: death, life-threatening situation, significant or sustained incapacity or disability,
16
17 338 hospitalization, prolongation of hospitalization, deformity or birth defect, potentially serious
18
19 339 event. Any SAE or event of interest, regardless of its causal relationship with the trial
20
21 340 treatment or the research, must be reported to the sponsor, as soon as possible and the latest
22
23 341 24 hrs after their occurrence for SAE. The investigator is in charge of noting and reporting all
24
25 342 SAE occurring during the study, from the date of the signed informed consent, during the
26
27 343 study's expected duration of follow up (6 months after the treatment cessation for women and
28
29 344 at age 6 months for newborns). Furthermore, regardless of the event occurrence deadline after
30
31 345 the end of the study, all SAE that may be due to the research must be declared to the sponsor
32
33 346 when there is no other cause and effect accounted for (such as serious events that may occur
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35 347 long after exposure to the drug, such as cancers or congenital abnormalities).
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43 348 Some AE and SAE are expected and can be linked to i) pregnancy, ii) the disease (occurrence
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45 349 of another miscarriage) or study treatment (benign, adverse reaction AR, as digestive
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47 350 symptoms or severe, serious adverse reaction SAR as skin reaction, which requires to
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49 351 immediately stop the treatment).
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56 353 ***Independent Data and safety monitoring Board (IDSMB)***
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3 354 The IDSMB comprises 8 members without competing interests, not directly involved in other
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5 355 aspects of the trial and independent from the sponsor: 7 voting members, experts in
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7 356 complementary fields of the pathology and clinical trials (internal medicine, methodology,
8
9 357 specialist of teratogenic agents, neonatology, hematology, ophthalmology) and a non-voting
10
11 358 independent statistician. IDSMB monitors the data that affects patient safety, provides
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13 359 independent expertise for the evaluation of adverse events (AE) occurring during the study,
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15 360 expresses an opinion concerning the benefit / risk ratio and provides recommendations in
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17 361 order to help the steering committee to take decisions on protocol modification or early
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19 362 termination of the study. The members of the IDSMB will have to meet every 20 inclusions.
20
21
22 363 The IDSMB will transmit its recommendations to the steering Committee, which decides
23
24 364 whether or not to stop the study. Given the safety data and the treatment benefit, several
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26 365 different recommendations can be provided by the IDSMB: i) continuation of study without
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28 366 protocol modification, ii) continuation of study with modification of protocol, iii) temporarily
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30 367 discontinuation of inclusions, iiiii) early termination of the study
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36 368 Over the course of study, the IDSMB will have an advisory role in reassessing the benefit/risk
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38 369 ratio of the study.
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371 *Number of patients*

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48 372 The total number of scheduled patients to be recruited is 300, on the basis of the following
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50 373 hypotheses:

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53 374 ➤ 80% of the included women should get pregnant (ALIFE study).¹¹
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55 375 ➤ Among women who will get pregnant, we suppose that the rate of achieved
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57 376 pregnancy will be of 70% in the placebo group (PREFIX study).¹⁴
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3 377 To demonstrate a minimal clinically important difference of 15% in live-birth rates on HCQ
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5 378 (i.e. a 50% relative risk reduction in pregnancy loss), we need to enroll 300 women for a
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7
8 379 power of 80%, and a two-tailed alpha risk of 5%.

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11 380 The anticipated duration of recruitment is 3 years and the duration of participation of each
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13 381 patient: from 7 to 29 months.

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19 383 *Analyses plan*

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22 384 Analyses will be firstly conducted on an intention to treat basis for all women who have
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24 385 started pregnancy. Hierarchical analysis including three steps is planned :comparison of the
25
26 386 number of live and viable births, between the two treatment groups i) among all the women,
27
28 387 ii) among women without known APL antibodies, iii) among women without known
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30 388 thrombophilia (APL or inherited thrombophilia). We plan to adjust for age and number of
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32 389 previous losses if the difference between the two treatment groups is statistically significant (p
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34 390 $< .05$).

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39 391 Secondary analyses will be conducted on an intention to treat mode:

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42 392 For all included women, comparison of the number of live and viable births between the two
43
44 393 treatment groups.

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48 394 Among women who will get pregnant:

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51 395 ➤ Measure of treatment effect in women at higher risk of further miscarriage,
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53 396 according to the presence of thrombophilia, past history of intrauterine fetal
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55 397 death after 10 week's gestation, past history of miscarriage, > 3 previous
56
57 398 miscarriage or, no previous newborn baby,

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3 399 ➤ Comparison between the two treatment groups of the occurrence of several
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5 400 obstetrical events (miscarriage and intrauterine fetal death, vasculo placental
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7 401 disease, premature birth),
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9
10 402 ➤ Comparison between the two treatment groups of newborn clinical data
11
12 403 (gestation time at delivery, birth weight adjusted to the gestation time, number
13
14 404 of newborn babies living at 28 days and congenital abnormalities.

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18 405 A "per-protocol analysis" (primary endpoint) will include only patients properly exposed to
19
20 406 the study treatment, that is i) at least during a whole menstrual cycle before pregnancy onset
21
22 407 and until 10 weeks of amenorrhea ii) compliance \geq 80%.

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25 408 No unblinded interim analysis is planned.

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28 409 The biological collection will be carried out by the CRB of the CHRU of BREST and
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30 410 integrated into the collection "thrombosis" declared to the Ministry (2008-214/ DC-2009-
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32 411 925).

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37 38 39 413 **DISCUSSION**

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43 414 To date, there has not yet been an optimal therapy with conclusive clinical benefit for
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45 415 unexplained RM, in the absence or presence of inherited thrombophilia. Providing some
46
47 416 psychological support seems to be the only available therapy for women with unexplained
48
49 417 RM. Although the probability of a further normal pregnancy seems high, (~70% at age 32
50
51 418 after 3 consecutive miscarriages),⁵⁸the proposed therapeutic interventions are sometimes
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53 419 excessive, (e.g. possible side effects) and costly (e.g. intravenous immunoglobulins, assisted
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55 420 procreation). Therefore, it is of utmost importance to investigate other therapeutic options.
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3 421 In RM women with high titers of APL antibodies but without any other previous clinical
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5 422 event listed in APS (thrombotic or obstetric event), benefit of antithrombotic treatment
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7 423 (aspirin or LMWH) remains controversial. Clinical benefit of HCQ has not been demonstrated
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9 424 in prospective studies with robust methodology.⁵⁹
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13 425 In conclusion, the benefit/risk ratio and low cost are strong incentives for assessment of HCQ
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15 426 in unexplained RM irrespective of maternal thrombophilic status.
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22 428 **ETHICS, REGULATORY APPROVALS AND DISSEMINATION**

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25 429 Agreement from the French National Public Health and Drug Security Agency (160765A-22)
26
27 430 and ethical approval from the Committee for the Protection of Persons of NORD-OUEST I
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29 431 (2016-001330-97) have been obtained on November 4th 2016 and March 2nd 2017,
30
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32
33 433 French National Health and Drug Safety Agency as EudraCT # 2016-001330-97. The
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37 435 Protocol version V3.0, November 30, 2017
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37 455 **Contributors**
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39
40 456 EP conceived and designed the study. EP drafted the original grant proposal and trial
41
42 457 protocol. LDSM provides methodological and statistical expertise. GM has assisted in
43
44 458 developing the protocol, helped with implementation and has responsibilities for day-to-day
45
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20 470 **Competing interests for all trial investigators** None declared.
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31
32 474 The data that will support the findings of this study will be available when the findings will be
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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym reported on page 1
Trial registration reported on pages 3, 21	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier reported on page 21
Funding	4	Sources and types of financial, material, and other support reported on p 9, 23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors reported on p 1, 22
	5b	Name and contact information for the trial sponsor reported on p 21
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities reported on p 23
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) reported on the study protocol (supplementary file) on pages:15-17, 58-61
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention reported on pages 5-9
	6b	Explanation for choice of comparators reported on p 6,7
Objectives	7	Specific objectives or hypotheses reported on page 10
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) reported on page 11

Methods: Participants, interventions, and outcomes

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4	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained reported on pages 11, 22
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8	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) reported on pages 12-13
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12			
13	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered reported on pages 14
14			
15			
16		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) reported on page 17 and on page 48 of the study protocol (supplementary file)
17			
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21		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) reported on page 46 of the study protocol (supplementary file)
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25		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial reported on page 15
26			
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28	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended reported on pages 16 and, on pages 33-34 of the study protocol (supplement)
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36	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) reported on page 15, 16 and on pages 34-44 of the study protocol
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41	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations reported on page 18-19
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45	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size reported on page 48 and 70 of the study protocol (supplementary file)
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Methods: Assignment of interventions (for controlled trials)

Allocation: reported on page 13-14 and on page 44 of the study protocol (supplementary file)

52			
53	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
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2	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central
3	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
4	mechanism		describing any steps to conceal the sequence until interventions are
5			assigned
6			
7	Implementation	16c	Who will generate the allocation sequence, who will enrol participants,
8			and who will assign participants to interventions
9			
10	Blinding	17a	Who will be blinded after assignment to interventions (eg, trial
11	(masking)		participants, care providers, outcome assessors, data analysts), and
12			how
13	reported on page 14		
14	and on page 44		
15	of the study protocol	17b	If blinded, circumstances under which unblinding is permissible, and
16	(supplementary file)		procedure for revealing a participant's allocated intervention during
17			the trial
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Methods: Data collection, management, and analysis

21	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other
22	methods		trial data, including any related processes to promote data quality (eg,
23			duplicate measurements, training of assessors) and a description of
24			study instruments (eg, questionnaires, laboratory tests) along with
25			their reliability and validity, if known. Reference to where data
26			collection forms can be found, if not in the protocol
27			reported on pages 68, 69 of the study protocol (supplementary file)
28		18b	Plans to promote participant retention and complete follow-up,
29			including list of any outcome data to be collected for participants who
30			discontinue or deviate from intervention protocols reported on p 48-49
31			of the study protocol
32	Data	19	Plans for data entry, coding, security, and storage, including any
33	management		related processes to promote data quality (eg, double data entry;
34			range checks for data values). Reference to where details of data
35			management procedures can be found, if not in the protocol
36			reported on pages 68-69 of the study protocol (supplementary file)
37	Statistical	20a	Statistical methods for analysing primary and secondary outcomes.
38	methods		Reference to where other details of the statistical analysis plan can be
39			found, if not in the protocol
40			
41	reported on pages 19-20 and on	20b	Methods for any additional analyses (eg, subgroup and adjusted
42	pages 62-64 of the study		analyses)
43	protocol		
44		20c	Definition of analysis population relating to protocol non-adherence
45			(eg, as randomised analysis), and any statistical methods to handle
46			missing data (eg, multiple imputation)
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Methods: Monitoring

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53	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role
54			and reporting structure; statement of whether it is independent from
55			the sponsor and competing interests; and reference to where further
56			details about its charter can be found, if not in the protocol.
57			Alternatively, an explanation of why a DMC is not needed
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reported on page 17-18 and on page
61 of the study protocol.

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2		21b	Description of any interim analyses and stopping guidelines, including
3			who will have access to these interim results and make the final
4			decision to terminate the trial reported on page 18 and on page 62 of the
5			study protocol (supplementary file)
6	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and
7			spontaneously reported adverse events and other unintended effects
8			of trial interventions or trial conduct reported on pages 16-17 and on pages
9			55-60 of the study protocol (supplementary file)
10	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and
11			whether the process will be independent from investigators and the
12			sponsor reported on page 66 of the study protocol (supplementary file)
13			
14			

Ethics and dissemination

15			
16			
17	Research ethics	24	Plans for seeking research ethics committee/institutional review board
18	approval		(REC/IRB) approval reported on pages 3, 21 and on pages 46,59,61
19			of the study protocol
20	Protocol	25	Plans for communicating important protocol modifications (eg,
21	amendments		changes to eligibility criteria, outcomes, analyses) to relevant parties
22			(eg, investigators, REC/IRBs, trial participants, trial registries, journals,
23			regulators) reported on page 66 and 70 of the study protocol (supplementary file)
24			
25			
26	Consent or assent	26a	Who will obtain informed consent or assent from potential trial
27			participants or authorised surrogates, and how (see Item 32)
28	reported on page		
29	67 of the study protocol	26b	Additional consent provisions for collection and use of participant data
30			and biological specimens in ancillary studies, if applicable
31			
32			
33	Confidentiality	27	How personal information about potential and enrolled participants will
34			be collected, shared, and maintained in order to protect confidentiality 70
35			before, during, and after the trial reported on page 65 of the study protocol
36			
37	Declaration of	28	Financial and other competing interests for principal investigators for
38	interests		the overall trial and each study site reported on page 23
39			
40	Access to data	29	Statement of who will have access to the final trial dataset, and
41			disclosure of contractual agreements that limit such access for
42			investigators reported on pages 64-65 of the study protocol (supplementary file)
43			
44			
45	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for
46	post-trial care		compensation to those who suffer harm from trial participation
47			reported on page 67 of the study protocol (supplementary file)
48	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to
49	policy		participants, healthcare professionals, the public, and other relevant
50			groups (eg, via publication, reporting in results databases, or other
51			data sharing arrangements), including any publication restrictions
52			reported on page 70 of the study protocol (supplementary file)
53		31b	Authorship eligibility guidelines and any intended use of professional
54			writers reported on page 70 of the study protocol (supplementary file)
55			
56			
57		31c	Plans, if any, for granting public access to the full protocol, participant-
58			level dataset, and statistical code
59			
60			

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates given as a supplementary file
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable reported on p 35, 39 of the protocol

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)" license.

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