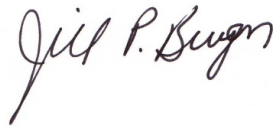


-407HUMAN SUBJECTS PROTOCOL**Protocol No. 11-00369****Title:** Preventive Approach to Congenital Heart Block with Hydroxychloroquine (PATCH)**Sponsor:** NIH**Principal Investigator:** Jill P. Buyon, MD.

New York University School of Medicine
 Hospital for Joint Disease
 Department of Medicine, Division of Rheumatology
 301 East 17th Street, Suite 1410, 14th Floor
 New York, NY
 10003
 Tel: 212-598-6110
 Email: jill.buyon@nyumc.org

Version date: August 6, 2019

P.I. Signature:

 Jill P. Buyon, M.D., Professor of Medicine
08/6/2019

Date

Purpose of the Study and Background**Purpose of the Study**

One of the strongest clinical associations with autoantibodies (Ab) directed to components of the SSA/Ro-SSB/La ribonucleoprotein complex is the development of congenital heart block (CHB) in an offspring, an alarming prospect facing 2% of primigravid mothers with these reactivities [reviewed in 1]. The risk is 10-fold higher in women who have had a previously affected child. Once 3rd degree block is identified, permanent reversal has never been achieved. Despite the attempts of large multicenter studies to forestall disease by careful monitoring, irreversible block and extensive myocardial injury have been documented within 7 days of a normal rhythm and PR interval. CHB is associated with a significant mortality and morbidity. Current prophylactic and treatment strategies include maternal steroids, plasmapheresis, sympathomimetics, and *in utero* cardiac pacing but none have significantly altered mortality, justifying the need for continued research. Two recent studies [2,3] utilizing an identical protocol of IVIG demonstrated that 1) this intervention does not prevent the recurrence of CHB 2) the recurrence rate of 17-18% is robust and reproducible 3) recruitment of patients is feasible. During the time period of the IVIG trials, basic science exploring the pathogenesis of disease supported the notion that macrophage Toll Like Receptor (TLR) signaling following ligation of the ssRNA complexed to the Ro protein contributes to cardiac fibrosis [4]. TLR signaling and fibrotic endpoints could be abrogated by chloroquine (CQ), which inhibits endosomal acidification. This *in vitro* observation was “translated” to patients by evaluating the use of hydroxychloroquine (HCQ) in an extensive retrospective chart review [5]. The combined data suggested a possible efficacy of HCQ, raising the consideration of this drug as a highly promising candidate for the prevention of CHB.

Specific Aim: To determine whether hydroxychloroquine use during pregnancy prevents the recurrence of CHB.

This will be approached in an open label Phase II trial entitled: **P**reventative **A**pproach to **T**herapy for **C**ongenital Heart Block with **H**ydroxychloroquine (PATCH), in pregnant women who have had a previous child with CHB. This is designed as an open-label trial employing Simon’s 2-stage optimal

design to allow for early stopping due to absence of treatment efficacy. The first stage requires 19 subjects, which are expected to be enrolled in the year of this award. Despite the rarity of disease and the requirement of a previous child with CHB, based on the US Research Registry for Neonatal Lupus (RRNL) and data from the UK, this proposal is feasible. If 3 mothers have a child with 2nd or 3rd degree CHB, the study is terminated after the first stage. Serial echocardiograms (monitor PR interval) and evaluation of biomarkers (HCQ levels, IFN α signatures, and Ab titers) will be part of the protocol. The results of this study should become an integral component of the counseling of women with anti-Ro/La Ab who are considering pregnancy.

Background

CHB associated with maternal anti-Ro Ab has a high mortality (20-30%, primarily fetal/neonatal) and morbidity (67% require permanent pacing before adulthood) [6,7]. In addition to conduction disease, 10-15% of affected offspring will have a life-threatening cardiomyopathy [8,9]. Histopathologic studies constitute a major basis for formulating hypotheses regarding the pathogenesis of CHB, which in turn guide the rationale for therapeutic approaches. Studies of several fetal hearts identified in utero with CHB or isolated myocarditis revealed that fibrosis is extensive and replaces the AV node, even in 20 and 22 week hearts [10], supporting the clinical observation that 3rd degree CHB is irreversible. The identification of a macrophage infiltration in fetuses dying most proximate to the time of diagnosis suggests that inflammation may be integral to the development of fibrosis [10].

Prenatal Considerations

From an immunologic perspective, reduction of a generalized inflammatory response has traditionally been considered a logical approach to the prevention or treatment of CHB. The use of maternal oral dexamethasone has been popularized by several groups but its scientific merit is underwhelming, and the risks may be substantial [11-13]. The PRIDE (PR Interval Dexamethasone Evaluation) in CHB study revealed no reversibility of 3rd degree CHB with dexamethasone, a higher mortality with dexamethasone (albeit these may have been sicker fetuses), and inconsistent effects on reversal of incomplete blocks [14].

Intravenous Immunoglobulin G (IVIG): A prospective study in which feasibility of recruitment and reproducibility of the recurrence rate was demonstrated but prevention of CHB was not achieved:

The consideration of IVIG in the prevention of CHB was based on our working hypothesis of the pathogenesis of disease. Tissue injury in the fetus is presumed to depend on the Fc γ Rn-mediated transplacental passage of maternal IgG Ab [15]. Anti-Ro/La Ab, by binding to translocated antigens on the surface of apoptotic cardiocytes generated during remodeling of the conduction system and surrounding tissue, may inhibit the normal physiologic removal of these cells [16]. Uncleared opsonized apoptotic cardiocytes may be subsequently efferocytosed by infiltrating macrophages with release of pro-inflammatory and profibrosing cytokines which transdifferentiate cardiac fibroblasts to a scarring phenotype [17]. This scenario supported the consideration of prophylactic therapy with IVIG based on 2 presumed mechanisms of efficacy. The first relates to the saturation of Fc γ Rn by IVIG, which would be expected to decrease fetal exposure to anti-Ro/La Ab by accelerating IgG catabolism in the maternal circulation and by decreasing placental transport [18]. The second relates to the potential attenuation of anti-inflammatory responses by increasing the macrophage expression of Fc γ RIIB [19]. This would represent a downstream effect in the targeted organ.

Accordingly, a multicenter, prospective, open-label study in the U.S. was initiated [2] (Preventative IVIG Therapy for CHB, PITCH). Enrollment criteria included the presence of anti-Ro Ab in the mother, birth of a previous child with CHB/neonatal lupus rash, current treatment with 20 mg/day of prednisone, and <12 weeks pregnant. IVIG (400 mg/kg) was given every 3 weeks from gestational week 12 - 24. The primary outcome was the development of 2nd or 3rd degree CHB. Twenty mothers completed the IVIG protocol before the predetermined stopping rule of 3 cases of advanced CHB was reached. CHB was detected at 19, 20, and 25 weeks; none occurred following a prolonged PR interval. One of these 3 mothers had 2 previous children with CHB. One child without CHB developed a transient rash consistent

with neonatal lupus. Sixteen children had no manifestations of neonatal lupus at birth. No significant changes in maternal titers of Ab to Ro60, Ro52, or La48 were detected over the course of therapy or at delivery. There were no safety issues.

In parallel with enrollment of the PITCH study, a European study was initiated in December, 2004. We and the P.I., Dr. Munther Khamashta, had agreed on the protocol. The study was terminated after 3 recurrent cases of CHB were identified following enrollment of 15 mothers [3]. Combining data generated from the U.S. and the European PITCH studies, there were 6 (18%) recurrences in 33 women who had previous pregnancies complicated by CHB. Each study was originally designed to conclude inefficacy of IVIG if 6 cases of 54 were identified. However, since the chances of one further recurrence in the next 21 (if combined US and UK) was greater than 98% assuming the 18% recurrence rate observed, the trial was deemed futile and terminated.

Hydroxychloroquine: A potential prophylactic approach

Antimalarials are among the most frequently prescribed medications in SLE patients. The prevention of flares and reduction of mortality are associated with the use of these medications as supported by a recent systematic review of high quality published evidence [20]. Serious adverse events such as cardiotoxicity are rare, even after prolonged use. With regard to retinal toxicity, CQ appears to be more toxic than HCQ [20].

The use of antimalarials to prevent SLE flares during pregnancy has been addressed by several studies. In a small, randomized double-blind trial of 10 SLE pregnant patients receiving HCQ (surprisingly, dose not stated) compared to 10 on placebo, the drug was administered between 8 – 18 weeks of gestation [21]. In the active drug group, there were no flares of disease activity compared to 3/10 in the placebo group. Importantly, neither congenital abnormalities at birth nor ophthalmologic or auditory abnormalities were detected up to a minimum follow up of 1.5 years. A second study, non-randomized, compared three groups of pregnant patients: those continuously exposed to HCQ before and throughout pregnancy (N = 56), those with no exposure (N = 163), and those in whom HCQ was discontinued prior to or during the first trimester (N = 38) [22]. The patients who discontinued HCQ were reported to have a higher degree of SLE activity and an increased frequency of flares. Ophthalmologic and auditory tests were limited to routine infant care although the authors did not report any clinically significant fetal abnormalities attributable to HCQ exposure. A third study showed that discontinuation of HCQ at the onset of pregnancy was associated with increased lupus activity [23]. Our own data from the Prospective Predictors of pRegnancy Outcome: bioMarkers In Antiphospholipid Antibody Syndrome and Systemic Lupus Erythematosus (PROMISSE) study also suggested the benefit of HCQ. Specifically, 105 patients with SLE were taking HCQ throughout pregnancy [24]. Overall, mild/moderate and severe flares were infrequent (<10%) and maintaining HCQ during pregnancy appeared to be of benefit in preventing flares. One child was reported to have an abnormal audiology screen at birth, which was considered inconsequential upon subsequent testing.

Despite accumulating evidence supporting the prevention of SLE flares during pregnancy, some physicians may remain reluctant to prescribe HCQ given isolated case reports of auditory toxicity [25] and retinal toxicity [26] in fetuses exposed to HCQ, in addition to data from an animal model regarding retinal deposition [27]. Currently, the FDA lists both CQ and HCQ as pregnancy risk category C (safety in human pregnancy has not been determined). However, recent reviews of the literature suggest that, in particular, HCQ is safe to use during pregnancy [20,28,29]. None of the studies (inclusive of over 250 children) found an increased frequency of congenital malformation. In 58 children tested, maternal use of HCQ was not associated with either hearing or visual abnormalities [28]. Available ECGs revealed no differences with regard to duration of the PR or QTC intervals between unexposed or exposed children [28].

The rationale for HCQ rests on translation of the pathophysiology of CHB which likely involves a complex cascade linking antibody to ultimate scar. Dual receptor signaling via uptake of the anti-Ro/La immune complexes by FcγR on macrophages, and delivery of associated ssRNA to the endosomal compartment for ligation with Toll Like Receptors (TLR7/8) may be critical steps toward

conduction damage. Since endosomal TLR binds ligand at low pH, pharmacologic approaches to attenuate TLR-dependent readouts have utilized CQ and bafilomycin which interfere with acidification [30,31]. Our own laboratory has recently demonstrated that both macrophage transfection with non-coding ssRNA that bind Ro60 and an immune complex generated by incubation of Ro60-ssRNA with an IgG fraction from a CHB mother or affinity purified anti-Ro60 significantly increased TNF α secretion, an effect not observed using control RNAs or normal IgG. Dependence on TLR was supported by the significant inhibition of TNF α release by IRS661 (inhibits TLR7) and CQ. Fibrosis markers were noticeably increased in fetal cardiac fibroblasts after incubation with supernatants generated from macrophages transfected with ssRNA or incubated with the immune complex, an effect abrogated by IRS661 or CQ [4]. These in vitro findings generate the hypothesis that HCQ, which reduces the acidification required for optimal ligation of TLR and has been shown to be safe in pregnancies, as detailed above, may prevent cardiac tissue injury associated with antibodies to these ssRNA-complexed proteins.

Another potential mechanism favoring efficacy in preventing CHB, is that HCQ by virtue of reducing endosomal acidification might also decrease transplacental transport of maternal antibodies. This possibility is supported by the fact that maternal IgG is pinocytosed at the apical surface of the syncytiotrophoblast into an acid endosomal compartment containing FcRn, and migrates via transport vesicles to the basolateral surface of the cell, where at physiologic pH it uncouples from the receptor (15).

Study Design

Nineteen women in Stage 1 and an additional 51 women in Stage 2 (if reached, for a total of 70 subjects) meeting eligibility criteria will receive 400mg per day of HCQ beginning as soon as pregnancy is established and informed consent obtained. Mothers already on HCQ will remain on 400mg, or escalate to 400mg if on 200mg. The primary investigator, Jill Buyon, M.D., holds a U.S. Federal Drug Administration-issued IND (Investigational New Drug) number (112357) for HCQ.

A. Screening visit: Prior to the qualifying visit, the consent form will be reviewed and signed. Laboratory tests will include: CBC, liver-function tests (routine chemistries; albumin will be important to standardize the effect of physiologic hemodilution of pregnancy in gauging changes in autoantibody titers), urinalysis, and a 24 h collection for measurement of creatinine clearance and protein excretion as deemed appropriate. Serologic profiles will include: measurement of ANA, anti-DNA, C3 and C4. Blood will be drawn for baseline anti-Ro/La titer HCQ level, and IFN α activity. Only the former will be available prior to qualification for enrollment. In total, 35 ml of blood will be drawn at screening.

B. Qualifying Visit: This is done as soon as Ab status is established, which should take several days (ship overnight and assay in Dr. Buyon's laboratory is one day turnaround time; for Khamashta, it is equivalent). A detailed history and physical examination will be performed. The patient will either be given a prescription for HCQ 200mg, 2 tabs hs, and advised to see an ophthalmologist, or told to maintain dose if 400mg. For women who have SLE, data will be collected to calculate the Systemic Lupus Erythematosus Pregnancy Disease Activity Index (SLEPDAI). Rheumatologists will complete assessment forms, which will be reviewed by Drs. Buyon and Izmirly to address any effect of treatment on underlying SLE activity per se. Of note, Dr. Buyon has provided these forms for use in the ongoing NIH-supported PROMISSE trial.

C. Follow-up Assessments: Fetal echocardiograms will be performed after enrollment (16-18 weeks) and weekly thereafter until 26 weeks, then biweekly until 34 weeks. Fetal echocardiographic scans will be performed according to standard methods [14,33,39], recorded on VHS videotape or DVD/Optical Disc by the pediatric cardiologist or obstetrician, and sent to the core echocardiographic lab and quantified on data abstraction forms by Dr. Friedman. After the final fetal echo at 34 weeks, patients will continue with routine obstetric and rheumatologic care as appropriate. Neonates will have an EKG and echocardiogram at birth and at 1-year follow-up (it is acknowledged that this will be

out of the window of LFM award but not affect outcome since to date CHB has not occurred de novo after birth). Each woman will undergo routine obstetrical ultrasounds as per the treating obstetrician. Targeted Level 2 ultrasounds will be required between 21-24 weeks, and between 30-32 weeks to assess fetal well-being. Mothers enrolled in Europe will have their echocardiograms initially read by their primary cardiologists with the tapes then forwarded to Dr. Friedman.

Between 20-25 weeks and 30-35 weeks, and at delivery for all women, 20 ml of blood will be drawn (10 for serum, 10 for plasma and isolation of DNA). Cord blood will also be obtained. Both HCQ levels and IFN- α activity will be evaluated in each sample to serve both as a proxy for compliance and as a potential biomarker of efficacy, respectively. Samples will be sent overnight to Dr. Buyon's lab at each visit (screening, 2nd trimester, 3rd trimester, and delivery), rather than having each site process the bloods, which may be unduly cumbersome for busy obstetrical practices. For patients enrolled in Europe, samples will be stored in Dr. Khamashta's lab and shipped in batch. Serum samples will be divided into aliquots of 500 μ l: 2 will have sodium azide added (final concentration 0.1%) and be stored at 4°C; remaining samples will be stored without azide at -70°C to form the serum specimen bank available to other investigators with approved research protocols (as in the RRNL). Plasma will be similarly stored. DNA will be stored for future investigations as spawned by the clinical results.

Enrolled patients will continue usual care dictated by their underlying rheumatic disease.

Outcome Measures: The primary outcome is recurrence of advanced heart block: i.e., echocardiogram reveals 2nd or 3rd degree AV block. First-degree block [PR >150 msec, i.e., >(normal mean +3 SD)] [33,39] does not constitute a primary outcome unless it progresses to more advanced block. In these situations the mother will be fully counseled regarding morbidity and mortality of 2nd and 3rd degree block, and discussion regarding data on dexamethasone will be provided. Follow-up will be continued by the patient's treating physician. Secondary outcomes include: 1) Prolonged mechanical PR interval (>150 msec) is detected but does not progress to more advanced AV block. EKG at birth must confirm 1st degree AV block. It is also possible that a fetus developing 1st degree block on study medication might have developed more advanced block in the absence of study medication. The detection of 1st degree block will prompt discussion with the steering committee, treating physician and patient which may lead to treatment with dexamethasone. The mother will be informed of the PRIDE results in which 2 women elected to take dexamethasone and the block reverted to normal sinus rhythm [33]. However, the patient will be informed that we do not know the natural progression or regression of 1st degree block, and that 1st degree block may spontaneously reverse. The mother will continue in the study. 2) Any sign of myocardial injury, without change in cardiac rate or rhythm: a) shortening fraction <28% = 2 SD below normal mean or qualitatively reduced systolic function; b) cardio-thoracic ratio >0.33; c) hydropic changes; d) moderate/severe tricuspid regurgitation. 3) Echocardiographic densities consistent with EFE confirmed postnatally. 4) Fetal death not related to cardiac dysfunction. An autopsy with full evaluation of the heart will be encouraged but cannot be mandated. If AV block or evidence of a cardiomyopathy can be "proven," then these will provide the basis for final categorization. If not possible, the death will not be considered a recurrence rate but will be reported. 5) NL rash. 6) Prematurity (gestational age <37 weeks at birth. 7) Birth weight <10% in the context of gestational age. 8) Abnormal fluid collection.

Evaluation of Ab Levels: This will be done exactly as described for the IVIG trial, using recombinant proteins Ro60, Ro52, and La48 in an ELISA [2].

HCQ levels: This assay will be done in Dr. Costedoat-Chalumeau's laboratory in France. At each patient encounter, the interval between the last ingestion of HCQ and blood sampling is recorded. HCQ is assayed by HPLC with fluorometric detection as described [35] with minor modifications. The detection limit is 10ng/ml, and the between-day and within-day coefficients of variation are <8%.

IFN α activity: This assay will be done in Dr. Peggy Crow's laboratory and will utilize the WISH cell assay, as we have previously reported in anti-Ro positive mothers of children with neonatal lupus [40].

The reporter WISH cells are incubated with 50% patient plasma or serum for 6 hours. Total mRNA is purified from WISH cell lysates and cDNA synthesized from total mRNAs. Forward and reverse primers are used for the genes MX1, PKR, and IFIT1, which are highly and specifically induced by type I IFN. The relative expression of each of the 3 tested IFN-induced genes is calculated as a fold increase compared to its expression in WISH cells cultured with media alone. The ability of patient serum to cause IFN-induced gene expression in the reporter cells is then compared to the mean and SD induced by healthy donor serum. The number of SD above the mean of healthy donors for each gene is calculated. Data are expressed quantitatively as the sum of the number of SD of each of the three genes above the mean of healthy donors. The 3 transcripts chosen for measurement represent coordinate activation of the IFN- α pathway as would be expected after ligation of the type I IFN receptor, instead of measurement of only one IFN- α -induced transcript which may not accurately represent pathway activation. Patients are considered to have high type I IFN activity if either of the two following criteria are met: 1) 2/3 tested IFN-induced genes are expressed >1 SD above the mean of healthy donors, and at least 1 is greater than or equal to 2 SD above the mean of healthy donors, or 2) 1 IFN-induced gene is expressed greater than 4 SD above the mean of healthy donor sera. If neither criterion is met, the sample is considered to have low type I IFN activity. Two-sided Fisher's exact test (sum of small p's method for observed \geq expected) is used to analyze the categorical data, and Mann-Whitney non-parametric t-test is used to compare quantitative data. All evaluations will be done in batch (with the exception of screening anti-Ro/La) when the patient has completed the study.

Characteristics of the Research Population

Number of Subjects and Subject Recruitment

Stage 1, 19 subjects will be given HCQ. If fewer than 3 recurrences are observed, an additional 51 subjects will be treated in Stage 2 (Years 2-3, seeking a renewal and/or NIH funding). However, if 3 or more recurrences are observed after Stage 1, it will be concluded that HCQ is not effective in preventing CHB. Ultimately, HCQ will be considered efficacious for CHB if fewer than 6 CHB cases occur among 70 subjects evaluated on no potentially confounding medications.

While some subjects will be prescribed the study drug (HCQ) by the PI, Dr. Buyon, or a designated study team member (covered by Consent Form Version 1), many patients will be referred to the study from other sites. In these cases, patients' own doctors will take responsibility for prescribing the study drug, and patients will consent to have their bloods and medical records sent to the investigators at NYU for data and sample analysis (as delineated in Consent Form Version 2). Dr. Buyon or another study team member will discuss the study with each potential subject and complete the informed consent process prior to enrollment. All subjects (74 in total) will be considered enrolled at NYU and consented using NYU IRB-approved consent forms.

Gender of Subjects

Because the aim of this study is to determine whether hydroxychloroquine use during pregnancy prevents congenital heart block, only female subjects will be recruited.

Age of Subjects

18-45

Racial and Ethnic Origin

No subject will be excluded from participation in this study on the basis of race or ethnic origin.

Eligibility (Inclusion) Criteria

1) Mothers must have anti-Ro and/or anti-La Ab documented in the NYU immunology laboratory (CLIA-approved), which utilizes an ELISA (Immunovision) as well as reactivity on ELISA to at least one of three recombinant antigens (48La, 52Ro, 60Ro, JB laboratory). Dr. Khamashta will likewise

verify Ab in his laboratory. 2) Mothers must have a previous child with cardiac NL, defined herein as: the presence of heart block (1st, 2nd, or 3rd degree) documented by electrocardiogram (EKG), echocardiogram, pacemaker, or statement in the medical record, and/or; presence of cardiac injury, which specifically includes autopsy evidence of a mononuclear infiltrate in the endocardium, myocardium, and pericardium and/or EFE on echocardiogram always associated with cardiac dysfunction. In PITCH, we included women with a prior child with rash; however, recent data generated from the RRNL suggest that recurrence of CHB following rash is 11%, not 18% [34]. Thus, inclusion of previous rash could lead to a falsely lowered recurrence rate, and will therefore be excluded. 3) Intrauterine pregnancy ≤ 12 weeks. 4) Mother may be taking ≤ 20 mg prednisone because, in our experience, CHB has developed in the presence of this dose. 5) Mother may be asymptomatic, or have a rheumatic disease such as SLE or SS. Maternal health status has not been considered an influence on the development of CHB [1]. 6) Mother may or may not already be taking HCQ. This latter point was discussed with Dr. Nathalie Costedoat-Chalumeau, who has published extensively on measurement of HCQ [35,36]. While it might be optimal for the mothers anticipating enrollment in the study to all have been on HCQ prior to conception, this is impractical. Some may never achieve pregnancy and not want to take HCQ unless they conceive (especially those asymptomatic). On the other hand, women with SLE are likely to already be on HCQ and it would limit enrollment to exclude these patients if all must initiate HCQ only at enrollment in the first trimester. Although the accepted dogma is that HCQ requires several months for maximal efficacy in treating rheumatic disease, it is unknown whether this would apply to transplacental passage or fetal levels (which are impossible to measure). Dr. Costedoat-Chalumeau suggests that HCQ is probably a three compartment model which includes the circulation, tissues and cells. In the circulation, the half life is approximately 7 days and in the tissues, it is 40 days [37,38]. In Dr. Costedoat-Chalumeau's experience, steady state blood levels of HCQ are achieved in 4-6 weeks. Thus, dosing the mother no later than 10 weeks gestation should provide sufficient fetal exposure before the vulnerable period of CHB which is generally accepted to span 18-24 wks. Furthermore, the placenta has to be formed for HCQ to gain access to the fetus and it may be effective quickly for the biology we are considering.

Exclusion Criteria

1) Mother does not have Ab to Ro or La. 2) Identification of any of the following structural lesions considered causal for CHB, i.e., those that could account for block because of fibrous disruption between the atrium and AV node or due to absence of the penetrating bundles of the AV node: a) atrioventricular septal defects; b) single ventricle c) developmental tricuspid valve disease; d) L-transposition of the great arteries; e) heterotaxia. 3) Mother is taking >20 mg prednisone/day. Although unlikely to be preventative, higher doses will constitute an exclusion. The final point of intense discussion centered around whether another exclusion should be the use of HCQ in the first pregnancy in which CHB occurred. While one could argue that in these mothers HCQ was not effective and perhaps will not be again, this assumption remains speculative and thus prior absence of efficacy of HCQ will not constitute an exclusion criteria.

Vulnerable Subjects

This study will examine two groups of vulnerable subjects – pregnant women and their fetuses. Congenital heart block associated with maternal anti-Ro antibodies, the rare disease this study seeks to understand and ultimately prevent, affects the fetuses of mother who carry these anti-Ro antibodies. It is therefore essential to include these vulnerable populations in this study, as our ultimate goal is to protect these mothers and their children by understanding and ultimately preventing this devastating disease.

Methods and Procedures

Methods and Procedures

With over 400 families now enrolled in the RRNL and our success at rapid enrollment in PITCH, we are well positioned to enroll in the current study. Most of the women enrolled in the RRNL are of childbearing age, motivation is quite high, and based on review of overall data, it is likely that at least 20% will again become pregnant and available for this study. Importantly, PRIDE and PITCH solidified a referral base of obstetricians and pediatric cardiologists. Furthermore, Dr. Khamashta has agreed to participate given that all 19 patients must be recruited in one year. The DSMB for the U.S. based PITCH study (Dr. Ware Branch, maternal-fetal medicine, Dr. Bonnie Bermas, rheumatologist with expertise in pregnancy and women's health issues, and Dr. Joan Merrill, rheumatologist with expertise in design and monitoring of clinical trials in SLE) in conjunction with Drs. Jill Buyon, Peter Izmirly, Deborah Friedman (pediatric cardiologist), and Mimi Kim (statistician) have had extensive discussions regarding the results of PITCH and the design of this current study. With regard to a randomized trial comparing any drug with placebo, Dr. Kim prepared the following analysis:

Assumptions 1. Recurrence rate of CHB (whether previous child had CHB or rash) is approx 19%.
2. Clinically meaningful outcome would be at least 50% reduction in recurrence rate.

Power Calculations 19% to 10% recurrence rate: 261 per group (N = 522)
19% to 5% recurrence rate: 97 per group (N = 194)

Data Analysis And Data Monitoring

Statistical considerations for overall study:

The proposed design has the following operating characteristics. Let p_1 = recurrence rate definitely not worthy of further investigation; p_2 = recurrence definitely worthy of further investigation. In this study, we define $p_1 = 18\%$ [41] and $p_2 = 5\%$. According to Simon's optimal 2-stage approach [32], the expected sample size is 29.8 subjects with a probability of early termination of 0.69 when the recurrence rate is equal to p_1 . The probability of accepting the treatment for further study is at most 5% if the recurrence rate is unacceptably high ($>18\%$) with that treatment. In contrast, there is a 90% probability of accepting the treatment for further study if the true recurrence rate is $\leq 5\%$. It is important to emphasize that the purpose is to obtain critical data on the efficacy of HCQ in the prevention of recurrent CHB and determine whether this preventive approach should be studied further. At study completion, the proportion of mothers having a child with advanced CHB will also be computed, along with corresponding 95% confidence intervals. Secondary endpoints that are binary (i.e., occurrence of 1st degree block, abnormal fluid collection) will be analyzed by similar approaches. Continuous variables (e.g., shortening fraction, gestational age) will be summarized by estimating means and standard deviations. The effect of therapy on HCQ levels, IFN- α signatures, and Ab titers will be evaluated by fitting mixed effects linear models to repeated assessments of titers obtained during follow-up. In addition, change in any of these analytes at specific time points from baseline will be analyzed using the paired t-test or Wilcoxon signed rank test, depending on distribution of the data.

Safety committee. An independent DSMB will convene by teleconference three times during the year. The members include Drs. Joan T. Merrill, Ware Branch, and Bonnie Bermas (all of whom served in PITCH).

Data Storage and Confidentiality

Data management. Study information will be recorded at the patients' obstetrical and rheumatology sites. Standardized, computer-adapted case report forms (CRFs) have been developed. Within 2 weeks of each visit, completed CRFs and tapes of the echocardiograms will be sent to Dr. Friedman. Following review, CRFs will be faxed to the coordinator at NYU for final error checks and entered into the computer database. The coordinator will maintain a calendar for each patient, monitor completion

of data collection forms, and mail delinquency lists to investigators and Drs. Friedman and Buyon monthly. The coordinator will generate monthly reports summarizing accrual by institution and country; demographic and clinical characteristics; adherence to treatment and follow-up; and unexpected or severe complications of treatment. Raw data will be transferred to Dr. Kim for analysis. Separate CRFs for demographics, SLEPDAI (as appropriate), medications, physical exam, laboratory data, and adverse events will be sent within 2 weeks to the coordinator who will review each form with Drs. Buyon and Izmirly. Serious adverse events will be reported to the local IRB and the DSMB within 24 h. A database structure and computer data entry interface has been developed using database management software. Data entry procedures will include quality control features such as duplicate record entry and comparison for verification, logic checks and range checks, computer logging of record entry and editing, and automated back-up. The computer is protected with a password at the BIOS level. Data on patients enrolled in Europe will be forwarded to the coordinating center at NYU.

Blood collected as part of this study will be stored for future research

SPECIMEN BANKING

1. The samples will be labeled with anonymous and unique codes generated by the FreezerWorks database.
2. In the FreezerWorks database, only authorized users with password will be able to access PHI. Paper charts will be kept in a locked room in MSB room 611.
3. Samples will be stored for future use for an indefinite time period.
4. The samples will be stored in MSB room 606 and Dr. Buyon will have access to them.
5. The type of research that will be done on stored samples at this time is not known at this time.
6. Genetic testing will not be done on these samples.
7. If a subject requests withdrawal of sample(s) the samples will be appropriately discarded and confirmation of this will be provided to the subject.

Risk/Benefit Assessment

Risk

The potential physical risks and discomforts to you which may relate to the treatment with hydroxychloroquine are discussed below.

Hydroxychloroquine is part of a group of medications initially used to treat malaria, but usage has expanded to rheumatologic conditions such as systemic lupus and rheumatoid arthritis. Even though the U.S. Food and Drug Administration (FDA) lists hydroxychloroquine as a medication that has not been determined to be safe in pregnancy, extensive review of the medical literature suggests that this drug is safe during pregnancy. Results from animal studies showed that hydroxychloroquine could have visual side effects on the baby and there are also isolated case reports of hearing and visual problems in babies whose mothers received this drug during pregnancy. However, none of the clinical studies (inclusive of over 300 children) using hydroxychloroquine during pregnancy have found a higher frequency of hearing problems, visual problems or any other malformation in the babies exposed to the medication during pregnancy than in pregnancies in which the baby's mother did not take hydroxychloroquine. The potential side effects for the mothers are rare and consist of temporary gastrointestinal discomfort, discoloration of the skin and visual problems. A patient taking this drug should be seen by an eye doctor once per year and therefore if you have never been on this drug and are taking it only for this study, you should see an eye doctor. Two pills each day is the dose of hydroxychloroquine used for this study and both pills can be taken together.

With regard to having blood drawn, minor bruising at the site of blood drawing is common. Less common are larger bruises, inflammation of the vein where the blood was drawn, infection, and blood clots. Rarely, people faint from having blood drawn. We will draw your blood while you are sitting or lying on your back and ask you to remain in that position for several minutes to further minimize the risk of fainting. The risk of removing the amount of blood required for the study is minor. The additional amount of blood drawn only for this study is not large (1 tablespoon at each visit) and does not pose an additional risk to you or your unborn child.

No independently confirmed adverse effects on the fetus resulting from prenatal diagnostic ultrasound exposure have been reported. Acoustic output (the amount of sound applied to the mother's abdomen to produce the ultrasound image of the fetal heart) will be kept below acceptable limits.

Protection Against Risks

Safety committee. An independent DSMB will convene by teleconference three times during the year. The members include Drs. Joan T. Merrill, Ware Branch, and Bonnie Bermas (all of whom served in PITCH).

Individual stopping rules: Fetal: 1) Development of advanced block (2nd or 3rd degree); 1st degree block will not constitute a formal stopping point. 2) Decrease of amniotic fluid index <5 cm. 3) Intrauterine growth restriction (IUGR) - no fetal growth over 2 weeks. 4) Hydrops or myocardial dysfunction or EFE. 5) Death. Maternal: 1) Serious reaction to study drug, e.g. severe rash or intractable G.I. intolerance. 2) Withdrawal of consent. If isolated 1st degree heart block occurs, dexamethasone may be offered, and the patient may continue in the study. If a patient achieves an individual stopping point due to CHB and hydropic changes, she will be offered dexamethasone and the outcome of the pregnancy will be prospectively followed.

Study stopping rules: Enrollment is stopped if 3 fetuses develop 2nd or 3rd degree block.

Potential Benefits to the Subjects

We hope that this study shows that treatment with hydroxychloroquine prevents the development of heart block in your baby.

The potential benefits are substantial in relation to the small risks posed by participation.

This study is important because there is information from basic science research suggesting that hydroxychloroquine may be effective in preventing the inflammation and fibrosis which may result in heart block. This is only testing done with test tubes, not animals or humans. There is also a large study that reviewed the charts of many women with anti-Ro antibodies and this study found that hydroxychloroquine may be beneficial. However, we also are not sure whether for those mothers who received hydroxychloroquine, they may have had normal babies even without taking the drug. The results of this study will be used not only for a potential benefit to your own baby but also as a guide to help other pregnancies in which the baby is at risk for heart disease.

Subject Identification, Recruitment and Consent/Assent

Method of Subject Identification and Recruitment

With over 400 families now enrolled in the RRNL and our success at rapid enrollment in PITCH, we are well positioned to enroll in the current study. Most of the women enrolled in the RRNL are of childbearing age, motivation is quite high, and based on review of overall data, it is likely that at least 20% will again become pregnant and available for this study. Importantly, PRIDE and PITCH solidified a referral base of obstetricians and pediatric cardiologists. Furthermore, Dr. Khamashta has agreed to participate given that all 19 patients must be recruited in one year.

Innovative Plans for recruitment: 1.) Recruiter for patients: Sterling Larned is a mother enrolled in the RRNL and has agreed to participate in this project. She has two children with CHB and moderates Heart Block Kids (<http://health.groups.yahoo.com/group/heartblockkids/>), a support group for parents of children affected by neonatal lupus. The group will organize forums and educational panels to address CHB mothers' inquiries regarding planning pregnancy and participating in preventive trials and existing therapies. This approach allows us to create a link with an established group of mothers actively seeking counsel who are motivated to participate. 2.) Conference Call for Patients: This will take place every 3 months between Drs. Buyon, Izmirly, and Friedman, and patients who would like information about participation in the study.

Process of Consent

Each subject meeting the above-described eligibility requirements (or the authorized representative of the subject) will have the opportunity to review a consent form specific to this study and approved by New York University School of Medicine's Institutional Review Board. Either the P.I. of this study, Jill Buyon, M.D., or another individual authorized to obtain consent will review the consent form with each subject and will answer any questions the subject may have. Subjects will give their consent in writing by signing their name in the appropriate sections of the consent form, and this will only be done once the P.I. and/or other study personnel believes that the subject truly understands the significance of what they are signing.

Subject Capacity

All subjects will have the capacity to give informed consent to participate in this study.

Subject/Representative Comprehension

The initials and/or signatures at the appropriate sections of the consent form will provide the indication that the subject or subject's authorized representative has understood the information presented. The opportunity that will be provided each subject to review the consent form with authorized study personnel and to address any questions or concerns she may have will provide further assurance that the signatures and initials on the consent form indicate true "consent."

Debriefing Procedures

Not applicable.

Consent Forms

A consent form has been submitted. The P.I. is responsible for ensuring that valid consent is obtained and documented for all subjects.

Costs to the Subject

It will not cost subjects anything to participate in this study. Subjects (or their insurance company) will be charged or held responsible for the costs of their routine care (the care they would have received if they were not in this study).

Any study-related costs associated with subjects being in this study that are not part of their routine care will be paid by study funds.

This study is being sponsored by a grant. Portions of Dr. Buyon's and her research team's salaries are being paid by this grant.

Payment for Participation

You will not receive any reimbursement for participating in this study.