STATISTICAL ANALYSIS PLAN

STUDY TITLE:

Strategy to Prevent the Onset of Clinically-Apparent Rheumatoid Arthritis (StopRA)

PROTOCOL NUMBER:

ARA08

SHORT TITLE:	StopRA
NCT#:	NCT02603146
COMPOUND #:	Hydroxychloroquine
SPONSOR:	Division of Allergy, Immunology, and Transplantation National Institute of Allergy and Infectious Diseases – NIH
PREPARED BY:	[redacted]

This document is confidential and proprietary to DAIT/NIAID. This study is being conducted in compliance with good clinical practice, including the archiving of essential documents. Acceptance of this document constitutes agreement by the recipient that no unpublished information contained herein will be reproduced, published, or otherwise disclosed without the prior written approval of DAIT/NIAID, except that this document may be disclosed to appropriate Institutional Review Boards under the condition that they keep the information confidential.

ACKNOWLEDGEMENT AND SIGNATURE SHEET

Approved: _____ Date: _____
Kevin Deane, MD, PhD
Protocol Chair

Approved: _____ Date: _____
[redacted]
Medical Monitor

Approved: _____ Date: _____
[redacted]
Scientist

Approved: _____ Date: _____
[redacted]
Statistician

VERSION HISTORY

SAP Version	Version Date	Change(s)	Rationale
1.0	2023-05-08	Final version	

TABLE OF CONTENTS

LIST OF TABLES

1. LIST OF ABBREVIATIONS

Table 1: List of Abbreviations

Abbreviation	Term
ACPAs	Antibodies to Citrullinated Protein Antigens
ACR/EULAR	American College of Rheumatology/ European League Against Rheumatism
AEs	Adverse Events
Anti-CCP3	Anti-cyclic citrullinated peptide-3
BMI	Body mass index
CDAI	Clinical Disease Activity Index
CL-RA	Clinically Apparent Rheumatoid Arthritis
CRF	Case Report Form
CSR	Clinical Study Report
CTCAE	Common Toxicity Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DAS28-CRP	Disease Activity Score (28 joints) – C reactive protein
DMARD	Disease-Modifying Antirheumatic Drug
DSMB	Data and Safety Monitoring Board
EDC	Electronic Data Capture
EOS	End of Study
ESR	Erythrocyte Sedimentation Rate
FDR	First degree relative
GED	General Educational Development
HCQ	Hydroxychloroquine
HR	Hazard Ratio
hs-CRP	High-Sensitivity C-Reactive Protein
IA	Inflammatory Arthritis
IBW	Ideal Body Weight
ICH	International Conference on Harmonization
ID	Participant Identifier
IES	Intercurrent Event Strategy
IgM-RF	Immunoglobulin M – Rheumatoid Factor
IRB	Institutional Review Board

ITT	Intent-to-Treat Population
kg	kilogram
KM	Kaplan-Meier
LLN	Lower Limit of Normal
Max	Maximum
MDHAQ	Multi-dimensional Health Assessment Questionnaire
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
mITT	Modified Intent-to-Treat Population
mg	milligram
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
PLS	Population Level Summary
PP	Per Protocol Population
PRO	Patient Reported Outcome
PROMIS	Patient-reported Outcomes Measurement Information System
RA	Rheumatoid Arthritis
RAPID-3	Routine Assessment of Patient Index Data 3
RF	Rheumatoid Factor
SAEs	Serious Adverse Events
SAP	Statistical Analysis Plan
SD	Standard deviation
SDAI	Simple Disease Activity Index
SE	Shared Epitope
SOC	System Organ Class
SP	Safety Population
ULN	Upper Limit of Normal
WHO	World Health Organization
Y/N	Yes/No

2. PURPOSE OF THE ANALYSES

The purpose of this statistical analysis plan (SAP) is to describe the planned analyses and data displays to be included in the Clinical Study Report (CSR) for Protocol ARA08 StopRA. This document provides details on study populations, how the variables will be derived, how missing data will be handled and details on statistical methods to be used to analyze the safety and efficacy data.

The statistical analysis plan (SAP) is based on ICH guidelines E3 and E9 (Statistical Principles for Clinical Trials).

3. PROTOCOL SUMMARY

Title of the Protocol: Strategy to Prevent the Onset of Clinically-Apparent Rheumatoid Arthritis (StopRA)

ACE Protocol Number: ARA08

Protocol Chair(s): Dr. Kevin Deane, MD, PhD

Sponsor: DAIT/NIAID/NIH

Objectives:
The primary objective is to determine the efficacy of a 12-month course of hydroxychloroquine (HCQ) to prevent the development of clinically-apparent rheumatoid arthritis (RA) (as defined in Section 2.1, *Primary Objective)* at 36 months in subjects at high-risk for future RA due to high titer elevations of anti-cyclic citrullinated peptide-3 (anti-CCP3) (≥ 40 units) but who are without a history or clinical findings of inflammatory arthritis (IA) at Baseline.

Secondary objectives include:

1. To evaluate the safety of a 12-month course of HCQ in subjects who are at high-risk for development of RA.
2. To evaluate the impact of HCQ on development of clinically-apparent RA (as defined in Section 2.1, *Primary Objective*) in high-risk subjects 12 months after initiation of study treatment.
3. To evaluate the impact of HCQ on development of IA that may or may not meet criteria for RA in high-risk subjects 12 months after initiation of study treatment.
4. To evaluate the impact of a 12-month course of HCQ on the timing of development of clinically-apparent RA over the entire study period.
5. To evaluate the impact of a 12-month course of HCQ on the timing of development of IA, that may or may not meet criteria for RA, over the entire study period.
6. To explore the relationship between baseline and evolving symptoms[1], risk factors[2] and the development of future clinically-apparent RA and response to HCQ.
7. To evaluate the relationship between treatment with HCQ and amelioration of symptoms[1] of RA, and potential delay in onset of symptoms.
8. To explore underlying immune responses over time in the early natural history of RA development and in response to HCQ therapy through measurement of a variety of biomarkers.

Study Arms:

- Hydroxychloroquine: These subjects will receive 200 - 400 mg of HCQ (1-2 pills), based upon ideal body weight (IBW) at Screening, daily for 12 months.
- Placebo: These subjects will receive 1-2 pills of placebo (based upon IBW at Screening) daily for 12 months.

Study Design: This is a phase 2 multi-center, randomized, placebo-controlled, double-blind, parallel group 36-month clinical trial to evaluate the effectiveness and safety of intervention with a 12-month course of HCQ to prevent the future onset of clinically-apparent RA (See definition in Section 2.1, *Primary Objective*). At screening, study subjects will be without IA, but will be at high-risk for developing future RA within the trial period as indicated by elevated anti-CCP3 antibodies that are ≥40 units (that is a level ≥2 times the normal cut-off of ≥20 units). Two-hundred eligible subjects will be randomized in a 1:1 ratio to receive either self-administered HCQ or placebo.

Subjects will provide informed consent prior to any Pre-Screening or screening procedures. Subjects who are found to be eligible after the screening evaluation will return for a Baseline/Randomization visit within 30 days

[1] Baseline RA symptoms include self-reported joint pain, stiffness, and swelling, and overall fatigue.
[2] Risk factors include but are not limited to age, sex, genetic factors, socio-economic status, education, tobacco exposure, medications and medical hormone use, and dietary factors.

Title of the Protocol: Strategy to Prevent the Onset of Clinically-Apparent Rheumatoid Arthritis (StopRA)
of the initial screening visit. Subject eligibility will be confirmed prior to randomization. Eligible subjects will be randomized to receive either 200 - 400 mg (1-2 pills) of HCQ or 1-2 pills of placebo daily for 12 months based upon IBW at Screening. The weight-based dosing regimen for the study is outlined in Section 5.2, *Dosage Regimen*. Subjects will return to the study site for planned evaluations at Week 6, 12, 24, 36, and 52 (End of Treatment), and Months 18, 24, 30, and 36 (End of Study). During these study visits, subjects will have a joint exam and a physical examination. Study personnel will record the subject's interval medical history, assess adverse events, and collect samples for safety and mechanistic assessments (see Tables 6.1 & 6.2, *Schedule of Events*). Information on demographics (including socio-economic status and education), and other factors that may influence autoimmunity (e.g. tobacco exposure, hormonal status and exposures) may also be collected. Site coordinators will call subjects at Week 18, 30, and 42 and at Month 15, 21, 27, and 33 to answer subject questions, update contact information, and to assess AEs/reactions, study drug dosing and pregnancy status (during the treatment period), and joint symptoms. If a subject indicates that he/she is experiencing joint symptoms suggestive of RA (that include new or worsening joint pain, stiffness or swelling since the prior study visit), or symptoms suggestive of an AE, the subject will be asked to return to the study site as soon as possible. Visits and assessments for subjects who develop RA, IA with erosions, or who become pregnant prior to the Month 36 visits will be different from subjects who never develop RA. Details of these assessments may be found in protocol sections 6.5.9, *Evaluations Triggered by a Swollen Joint,* 6.5.10, *Procedures for Subjects Diagnosed with Inflammatory Arthritis or Rheumatoid Arthritis by an Outside Physician,* and 6.5.11, *Special Considerations for Pregnant Subjects*.
Endpoints: The primary efficacy endpoint is the development of clinically-apparent RA by 36 months, where clinically-apparent RA is defined in Section 2.1, *Primary Objective*. Secondary efficacy and safety endpoints are described in Sections 3.3.1, *Secondary Efficacy Endpoints,* and 3.3.2, *Secondary Safety Endpoints.*
Sample Size: 200 eligible subjects will be randomized in a 1:1 ratio.
Data Analyses: For the primary analysis, we are interested in demonstrating a long-term impact of a 1-year course of HCQ treatment on preventing the development of clinically-apparent RA (defined in Section 2.1, *Primary Objective*) in high-risk subjects. As such, rather than comparing full survival curves between treatment arms, the sample size for this study was selected to achieve sufficient power to compare survival curves at a fixed point 3 years after initiating treatment with HCQ. All secondary analyses will be conducted in an exploratory fashion with p-values and confidence intervals presented as descriptive statistics with no adjustments for multiple comparisons. Tests will be two-sided and interval estimates will be generated at the 95% confidence level.

4. GENERAL ANALYSIS AND REPORTING CONVENTIONS

The following analyses and reporting conventions will be used:

- Categorical variables will be summarized using counts (n) and percentages (%) and will be presented in the form "n (%)." Percentages will be rounded to one decimal place.
- Numeric variables will be summarized using n, mean, standard deviation (SD), median, minimum (min), maximum (max).
    - The median, min and max will be reported at the same level of significance as the original data.
    - The mean will be reported at one more significant digit than the precision of the data.
    - SD will be reported at two more significant digits than the precision of the data.
    - The median will be reported as the rounded average of the two middle numbers if the dataset contains an even number of observations.
- Test statistics including t and z test statistics will be reported to two decimal places.
- P-values will be reported to three decimal places if greater than or equal to 0.001. If less than 0.001, the value will be reported as "<0.001." A p-value can be reported as "1.000" only if it is exactly 1.000 without rounding. A p-value can be reported as "0.000" only if it is exactly 0.000 without rounding.

If departures from these general conventions are present in the specific evaluations section of this SAP, then those conventions will take precedence over these general conventions.

5. ANALYSIS POPULATIONS

Four analysis populations, the Intent-to-Treat (ITT), modified Intent-to-Treat (mITT), Per Protocol, and safety population will be defined for this study.

5.1. Safety Population

The safety population (SP), which will be used for all safety analysis, will include all participants for whom study treatment is initiated.

5.2. Intent-to-Treat Population

The Intent-to-Treat population (ITT) will include all randomized participants.

5.3. Modified Intent-to-Treat Population

The modified Intent-to-Treat (mITT) population will include all randomized participants who receive at least one dose of assigned study drug and meet entry criteria. The primary efficacy analyses will be based on the mITT population. Participants who, for whatever reason, do not complete their assigned therapy will be included in the mITT population in the groups to which they were randomized.

5.4. Per Protocol Population

The Per Protocol (PP) population will be defined as those participants in the mITT population who receive at least 80% of the assigned study treatment regimen with no substantive deviations from protocol procedures that would impact evaluation of efficacy.

A data review panel that will include the Protocol Chair (Deane) and participants from DAIT and Rho, Inc., will evaluate deviations from the protocol including, for example, violations of entry criteria, departures from assigned treatment regimen, use of prohibited therapy or HCQ prescribed outside of the study, failure to complete study visits, or to complete visits within the specified visit windows. The participant ID numbers will be replaced with randomly generated ID numbers for the PP population review.

The ARA08 study conducted an interim analysis in April 2022 as part of the regularly scheduled DSMB review. The Interim Statistical Analysis Plan is provided in Appendix 14.1. Due to the evidence of futility in the interim analysis results, the DSMB recommended that the ARA08 study stop early, and that the treatment assignments be released to the participants. Since the ARA08 study treatment assignments will be unmasked prior to database lock, the data review panel evaluation of the participants to include and exclude from the PP population will take place after the treatment assignments have been unmasked.

Levels of HCQ analyzed from serum samples taken during the study treatment period will not be used in the masked data review panel's evaluations of deviations from the protocol. The panel may exclude participants from the PP population if protocol deviations would be expected to impact the primary efficacy endpoint. Primary and secondary efficacy analyses may be replicated on the PP population.

6. STUDY PARTICIPANTS

6.1. Disposition of Participants

The disposition of all enrolled participants will be summarized in tables and listed. Enrollment is defined as participants who have signed the informed consent.

The numbers and percentages of randomized participants who qualify for each analysis population, completed study treatment and completed the study will be presented by treatment arm and overall. For participants who did not complete study treatment, the reasons for early discontinuation of study treatment will be presented by treatment arm. For participants who did not complete the study, the reasons for early termination of study participation will be presented by treatment arm.

6.2. Demographic and Other Baseline Characteristics

Summary descriptive statistics for baseline and demographic characteristics will be reported for the ITT, mITT and PP samples by treatment group. Characteristics to be summarized include:

- Age
- Race (White, Black, Asian, Native Hawaiian or Other Pacific Islander, American Indian or Alaska Native, Other/Unknown)
- Hispanic ethnicity (Hispanic, Not Hispanic)
- Jewish ethnicity (Jewish, Not Jewish, Unknown)
- Sex (Male, Female)
- Body Mass Index (BMI)
- Recruitment Method (First Degree Relative (FDR), Clinic Patient, General Population)
- Participant Has a FDR with RA (Yes/No)
- Smoking Status (Smoker, Non-smoker)
    - For current smokers, baseline pack years at the current level will be computed as:

      The average # cigarettes per currently smoked * the average # years at this level
- Baseline Cigar Use (Yes/No)
    - Cigar Users: Average Cigars per Day
    - Cigar Users: Number of Years Used Cigars
- Baseline Tobacco Pipe Use (Yes/No)
    - Tobacco Pipe Users: Average Bowls per Day
    - Tobacco Pipe Users: Number of Years Used Pipes
- Baseline Chewing Tobacco or Snuff Use (Yes/No)
    - Chewing Tobacco or Snuff Users: Average Wads per Day

    - Chewing Tobacco or Snuff Users: Number of Years Used Chewing Tobacco or Snuff

- Baseline Electronic Cigarettes Use (Yes/No)
    - Electronic Cigarettes Users: Electronic Cigarette Use Frequency (≤1 Day a Week, 2-4 Days a Week, ≥5 Days a Week)
    - Electronic Cigarette Users: Number of Years Used Electronic Cigarettes
- Baseline Anti-CCP3 (units)
- Baseline Anti-CCP3 Positivity (Negative (< Upper Limit of Normal (ULN), Low Positive (1.0 - <2.0 x ULN), Medium Positive (2.0 - <3.0 x ULN), High Positive (≥3.0 x ULN))
- Baseline IgM-RF (U/mL)
- Baseline IgM-RF Positivity (Negative (< Upper Limit of Normal (ULN), Low Positive (1.0 - <2.0 x ULN), Medium Positive (2.0 - <3.0 x ULN), High Positive (≥3.0 x ULN))
- Baseline hs-CRP (mg/L)
- Baseline hs-CRP Positivity (≤ 3.0 mg/L, > 3.0 mg/L)
- Baseline Shared Epitope (SE) Positivity (Negative on all epitopes and specific alleles (e.g. *0401,*0404, etc.), Positive on at least 1 epitope or specific allele)
- Baseline Shared Epitope Number of Positive Results (0, 1, 2, etc.)
- Baseline Pain Score
- Baseline Fatigue Score
- Baseline Morning Stiffness Duration
- Baseline Number of Tender Joints from Physician Joint Exam
- Baseline Disease Activity Score (28 joints) – C reactive protein (DAS28CRP)
- Baseline Routine Assessment of Patient Index Data 3 (RAPID3)
- Baseline Simple Disease Activity Index (SDAI)
- Baseline Clinical Disease Activity Index (CDAI)
- Baseline Patient-reported Outcomes Measurement Information System (PROMIS) Profile 29 Physical Health Score
- Baseline PROMIS Profile 29 Mental Health Score
- Baseline PROMIS Profile 29 Social Health Score

A listing of the demographic and baseline characteristics of randomized participants by treatment arm will be prepared.

6.3. Prior and Concomitant Medications

Medications will be coded according to the World Health Organization (WHO) Drug Dictionary (version V2016.01). Medications reported on the case report form (CRF) will be categorized for analysis as prior, concomitant, or post-treatment by comparing the medication start and stop dates with the first and last dose dates of study treatment. Medications that the participant had taken from 12 weeks prior enrollment through the end of study participation were recorded in the CRF. Prior medications will have both the medication start and stop dates prior to the date of the first dose of study treatment. Post-treatment medications will have the medication start date after the date of the last dose of study treatment. All other medications will be classified as concomitant, indicating that use of the medication overlapped with use of the study treatment by at least one day.

A listing of all medications reported in all participants in the safety population by treatment arm will be prepared.

6.4. Medical History

Medical histories are captured at screening for all randomized participants in the Medical History CRF. Body systems classified as abnormal in the Medical History CRF will be listed by treatment arm for all participants in the safety population.

7. STUDY OPERATIONS

7.1. Protocol Deviations

Major protocol deviations occurring throughout the duration of the study are captured on the Protocol Deviations CRF.

Major protocol deviations will be summarized and listed. The deviations will be summarized by whether the deviation was site-level or participant-level and by type of deviation:

- IRB Approval/Regulatory
- Informed Consent
- Entry Criteria Violations
- Study Treatment or Study Drug Related
- Protocol Mandated Concurrent Therapy Related
- Adverse Event/Serious Adverse Event Reporting Procedures
- Assessments and Laboratory Procedures
- Blind Break

The deviations will be listed by site, site-level deviations and participant level deviations, and by date of occurrence.

7.2. Randomization

Participants were randomized in a 1:1 ratio to either HCQ or placebo. Anticipating that some study sites would only randomize a few participants, an adaptive randomization procedure based on Pocock and Simon [**1**] minimization concepts was used to increase the likelihood of balance between treatment arms on key factors associated with progression to clinically-apparent RA. The factors used in the adaptive randomization procedure were smoker status (smoker vs. non-smoker), study site, and method of recruitment (i.e., first degree relative (FDR), general population, or clinic patient). Participants with elevated anti-CCP levels that met assay positivity criteria were identified for this trial through three general approaches that included the following:

- Pre-screening FDRs of patients with RA.
- Health-fair, biobank, or other population-based pre-screening.
- Identification in rheumatology clinics of participants with ACPA positivity in the absence of IA.

These three participant pools provided a sampling of the types of participants for whom this preventative approach would be applicable.

To avoid possible issues of familial correlation, our strong preference was to randomize only 1 FDR per family. Participants were asked about participation of immediate family members during the consent process and during study participation. However, recognizing operational

barriers to accurately track family membership and our suspicion that multiples per family would be a rare occurrence, randomization of multiple FDRs per family was allowed.

7.3. Blinding

Clinical staff were blinded to the treatment assignments until completion of the study with the exception of an unblinded pharmacist. In addition, clinical staff members, including the investigators, did not have access to any mechanistic data, and mechanistic laboratory staff did not have access to any clinical results until completion of the study.

No participant treatment assignments were unblinded prior to the end of the study.

7.4. Measures of Treatment Compliance

Both the investigational drug that was used during the course of the study, as well as any remaining unused investigational drug, were required to be accounted for on a drug accountability record provided or approved by the study sponsor or its designee.

Study participants received 1-2 pills of either HCQ or 1-2 pills of placebo for 12 months with dosing based upon Screening IBW as outlined in Table 2.

Table 2: Weight-based dosing regimen for HCQ (and placebo)

Weight (kilograms based on IBW*)	Number of pills**
≤ 24.4 kg (ideal or actual body weight)	Excluded from trial
> 24.4 - < 47 kg	1 pill daily
≥ 47 kg	2 pills daily

*IBW based on the following calculation:
Males: IBW = 50 kg + 2.3 kg for each inch over 5 feet, or subtract 1kg for every inch under 5 feet.
Females: IBW = 45.5 kg + 2.3 kg for each inch over 5 feet, or subtract 1kg for every inch under 5 feet.
** Each 200 mg HCQ pill contains 155 mg of active drug

Study therapy was dispensed per the schedule in Table 3.

Table 3: Study Therapy Distribution

Participant IBW at Screening	Baseline	Week 6	Week 12	Week 24	Week 36
> 24.4 - < 47 kg	1 bottle	2 bottles	2 bottles	2 bottles	3 bottles
≥ 47kg	2 bottles	3 bottles	4 bottles	4 bottles	6 bottles

Each bottle of study medication dispensed was recorded on the Study Drug Accounting Log CRF, along with the date the bottle was dispensed, the date the bottle was returned, the number of pills returned, and the number of pills lost.

The number of pills taken from each bottle will be computed as the number of pills in the bottle (i.e., 50) minus any pills lost or returned. The total number of pills expected to be taken will calculated as the participant's treatment period duration (treatment end date – treatment start date + 1) multiplied by the number of pills the participant was expected to take each day (1 pill or 2 pills) based on the participant's Screening IBW.

For bottles that were not returned to the site, the total number of pills expected will be imputed from the date the bottle was dispensed until the date the next study drug bottle is dispensed, or until the participant's treatment discontinuation date, whichever occurs earlier. The total number of pills taken will be imputed under 2 scenarios:

- Case 1 (best): the number of pills taken is assumed to equal the number expected,
- Case 2: the number of pills taken is assumed to equal 50 (all pills in bottle).

Percent treatment compliance for each participant will be calculated as:

$$\%\ Compliance = \frac{Total\ \ number\ of\ pills\ taken}{Total\ number\ of\ pills\ expected}\ x\ 100$$

Compliance for individuals who failed to return bottles will be reviewed by the masked data review committee for exclusion from the PP population.

Study drug dosing will be listed by participant, visit and bottle. Information will include the date of dispense and return, number of pills returned, and number of pills lost.

8. EFFICACY EVALUATIONS

8.1. Overview of Efficacy Analysis Methods

8.1.1. Multicenter Studies

Study participants were consented at 15 study sites. The analyses of survival estimates at fixed points in time will not be stratified. Site and method of recruitment will be considered as possible fixed covariates in models for secondary efficacy analyses. All safety analyses will be based on pooled data with no adjustment or stratification.

8.1.2. Assessment Time Windows

Visit windows for each scheduled visit are provided in Table 4. The Baseline visit must be performed within 30 days of the Screening visit.

Table 4: Visit Target Days and Windows for Scheduled Visits

Visits	Target Day	Visit Type	Window
Baseline	0	Clinic	≤30 days from Screening
Week 6	42	Clinic	±7 days from target
Week 12	84	Clinic	
Week 18	126	Telephone	
Week 24	168	Clinic	
Week 30	210	Telephone	
Week 36	252	Clinic	
Week 42	294	Telephone	
Week 52	364	Clinic	±14 days from target
Month 15	457	Telephone	
Month 18	548	Clinic	
Month 21	639	Telephone	
Month 24	731	Clinic	
Month 27	822	Telephone	
Month 30	913	Clinic	
Month 33	1004	Telephone	
Month 36	1096	Clinic	

Data collected outside of the visit window will not be removed from analyses categorized by visit and will be included in analyses where time is a continuous variable.

8.1.3. Timing of Analyses

Final analyses for all endpoints will be conducted after participants have completed study participation and all data collected are locked.

8.1.4. Multiple Comparisons/Multiplicity

This study has a single primary analysis to be tested at α=0.05. Consequently, no adjustments for multiplicity are needed for Type I error protection.

The secondary efficacy analyses are considered to be supportive with p-values and confidence intervals presented as descriptive measures of strength of evidence rather than formal statistical inference. Therefore, no multiplicity adjustments are needed for this study.

8.1.5. Intercurrent Event Strategies (IES) for Time to Event Analysis

For each time to event analysis, one of the intercurrent event strategies (IES) provided in Table 5 will be specified for the estimand.

Table 5: Intercurrent Event Strategies (IES) for Time to Event Analyses

IES Policy 1	IES Policy 2 (Note: Differences from Policy 1 are in blue text.)
• Participants who terminate early from the study prior to *endpoint* will be censored at the time of early termination.	• For participants who terminate early from the study prior to *endpoint* and have consented to be contacted via phone, o a participant-reported diagnosis of RA that was treated will be counted as a case of CL-RA/IA at the time of initiation of the RA medications. o For participants without a reported RA diagnosis, data will be censored at the date of last phone contact. • For participants who terminate early from the study prior to *endpoint* and cannot be contacted, data will be censored at the time of early termination.
• Participants who choose to take off-study HCQ on a continuous basis prior to *endpoint* will be censored at the time this therapy began.[+]	• Participants who choose to take off-study HCQ on a continuous basis prior to *endpoint* will not be censored at the time this therapy began. [+]
• Participants who were diagnosed with RA by an outside physician and started prohibited medications will be censored at the time the medications were started, if started prior to *endpoint*.[*] Only cases where the data collected on-study (at a study visit) meet the study-defined *endpoint* definition will count as CL-RA/IA in the analysis.	• Participants who were diagnosed with RA by an outside physician and started prohibited medications prior to *endpoint* will be included in the analysis as having developed CL-RA/IA at the time the prohibited medications were initiated, regardless of whether or not the diagnosis can be confirmed using study-defined *endpoint* criteria and study data.[&]
• Participants who started prohibited medications during study participation to treat conditions other than RA will be censored as follows: o If a participant is taking a prohibited medication other than corticosteroids, per protocol section 5.6, then time-in-study will be censored at the start of the first prohibited medication.	• Participants who started prohibited medications during study participation to treat conditions other than RA will be censored as follows: o If a participant is taking a prohibited medication other than corticosteroids, per protocol section 5.6, then time-in-study will be censored at the start of the first prohibited medication.

IES Policy 1	IES Policy 2 (Note: Differences from Policy 1 are in blue text.)
○ If a participant is taking corticosteroids, then time-in-study will be censored at the time when the permitted conditions in protocol section 5.5 are violated.	○ If a participant is taking corticosteroids, then time-in-study will be censored at the time when the permitted conditions in protocol section 5.5 are violated. • For participants who terminate early from the study prior to a diagnosis of RA and have consented to be contacted via phone, data for a participant who reports use of a prohibited medication to treat conditions other than RA will be censored at the time the prohibited medication was started.
• Participants who discontinue study-provided HCQ/placebo prematurely or who are not compliant with planned dosing will be included in the analysis and will not be censored at the time of study drug discontinuation or noncompliance.	• Participants who discontinue study-provided HCQ/placebo prematurely or who are not compliant with planned dosing will be included in the analysis and will not be censored at the time of study drug discontinuation or noncompliance.

+ Participants who took off-study HCQ (HCQ not provided by the study) continuously for at least 4 weeks prior to meeting the endpoint were censored at the time of starting the off-study HCQ

* Participants who were diagnosed with RA by an outside physician and did not start prohibited medications prior to endpoint were not censored at the time of the RA diagnosis.

& Participants who were diagnosed with RA by an outside physician and did not start prohibited medications prior to endpoint were not classified in the analysis as having developed CL-RA/IA.

8.2. Primary Objective

The primary objective for the ARA08 study is to determine the efficacy of a 12-month course of HCQ to prevent the development of clinically-apparent RA (CL-RA) at 36 months in participants at high-risk for future RA due to high titer elevations of anti-CCP3 (≥ 40 units) but who are without a history or clinical findings of IA at Baseline.

8.2.1. Computation of the Primary Clinical Endpoint

Individuals who have ≥1 swollen joint consistent with RA-like synovitis will be evaluated for presence of clinically-apparent RA.

The development of clinically-apparent RA (CL-RA) at 36 months is the primary clinical endpoint to evaluate the primary objective. CL-RA will be defined using the 2010 ACR/EULAR Classification Criteria as either:

(1) A score of ≥ 6 defining "definite RA" or

(2) A joint examination consistent with RA-like synovitis with ≥ 1 erosion identified via x-ray imaging of the hands, wrists, and feet.

At each clinic visit, a joint exam and physician's assessment are conducted. If a participant presents with swollen joint(s) that are consistent with RA-like synovitis, all the items necessary to establish the fulfillment of the 2010 ACR/EULAR criteria will be ascertained (See Table 6); however, the laboratory parameters including IgM-RF, anti-CCP3 and hsCRP will be tested at a central laboratory. Due to issues regarding sample stability and test reproducibility, hsCRP will

be used for the calculation of the ACR/EULAR criteria and disease activity measures; ESR will not be used.

Table 6: 2010 ACR/EULAR Classification Criteria

Who should be tested? Patients with ≥1 swollen joint consistent with synovitis not better explained by another disease. If the patient meets these initial criteria with a score of ≥6/10 they can be classified as having 'definite RA':

A. Joint involvement*	
1 large joint	0
2-10 large joints	1
1-3 small joints	2
4-10 small joints	3
>10 joints (at least 1 small)	5
B. Serology (at least 1 test needed)	
Negative RF and ACPA	0
Low positive RF or ACPA	2
High positive RF or ACPA**	3
C. Acute-phase reactants (at least 1 test needed)	
Normal CRP and ESR	0
Abnormal CRP or ESR	1
D. Duration of symptoms	
<6 weeks	0
≥6 weeks	1

* Joint involvement refers to any *swollen* or *tender* joint on examination. Distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal joints are *excluded from assessment*. "Large joints" refers to shoulders, elbows, hips, knees, and ankles. "Small joints" refers to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists.

** High positive is equivalent to >3 times the upper limit of normal based on the reference range of the laboratory that assesses the biomarker.

NOTE: Patients with erosive disease typical of rheumatoid arthritis with a history compatible with prior fulfillment of the 2010 criteria should be classified as having RA.

8.2.2. Primary Estimand of the Primary Clinical Endpoint

The primary estimand for the primary clinical endpoint of the development of clinically-apparent RA (CL-RA) at 36 months will be analyzed according the following specifications:

Table 7: Primary Estimand of Primary Objective

Variable: Time to development of CL-RA	
Estimand Label: Primary 1.0	
Population: mITT	**IES Policy 1**
Population Level Summary (PLS): Estimated risk at 36 months for each treatment arm and the difference between the treatment arms (with 95% confidence intervals) will be derived from Kaplan-Meier (KM) curves and using the Greenwood formula for estimating the variance.	**Analysis**: The analysis will be based on the KM estimated risk of CL- RA at 36 months, where risk = (1-KM estimated probability of "survival"). Survival for this analysis is defined as absence of CL-RA. Estimated risks will be derived from a Kaplan-Meier curve using censored time-to-event data to account for attrition under the assumption of non-informative censoring. The test statistic will be a Wald-type chi-square statistic derived by dividing the difference of the logit-transformed KM survival estimates for each arm by the associated variance derived using the delta-method. (Klein, et al. 2007)) [**2**]

8.2.3. Sensitivity Estimands of the Primary Endpoint

Three sensitivity estimands will be analyzed to support the evaluation of the primary objective.

Table 8: Sensitivity Estimands of Primary Objective

Variable: Time to development of CL-RA	
Estimand Label: Sensitivity 1.1	
Population: mITT	**IES Policy 2**
Population Level Summary: Same as Primary 1.0	**Analysis:** Same as Primary 1.0
Estimand Label: Sensitivity 1.2	
Population: PP	**IES Policy 1**
Population Level Summary: Same as Primary 1.0	**Analysis:** Same as Primary 1.0
Estimand Label: Sensitivity 1.3	
Population: PP	**IES Policy 2**
Population Level Summary: Same as Primary 1.0	**Analysis:** Same as Primary 1.0

8.3. Secondary Objectives

The secondary objectives for the ARA08 study and the analyses to evaluate them are provided in this section. Planned analyses described in the subsections below are based on the mITT

population. If warranted, analyses may be repeated on the PP population at the discretion of the study team.

8.3.1. Study Objective 2

Study Objective 2 is to evaluate the impact of HCQ on development of CL-RA in high-risk participants 12 months after initiation of study treatment. The clinical endpoint to evaluate this objective will be the development of CL-RA at 12 months. Details on the derivation of the development of CL-RA are provided in section 8.2.1.

Table 9: Estimand of Study Objective 2

Variable: Time to development of CL-RA	
Estimand Label: Secondary 2.1	
Population: mITT	**IES Policy 1**
Population Level Summary: Estimated risk at 12 months for each treatment arm and the difference between the treatment arms (with 95% confidence intervals) will be derived from Kaplan-Meier (KM) curves and using the Greenwood formula for estimating the variance.	**Analysis**: The analysis will be based on the KM estimated risk of CL-RA at 12 months, where risk = (1-KM estimated probability of "survival"). Survival for this analysis is defined as absence of CL-RA. Estimated risks will be derived from a Kaplan-Meier curve using censored time-to-event data to account for attrition under the assumption of non-informative censoring. The test statistic will be a Wald-type chi-square statistic derived by dividing the difference of the logit-transformed KM survival estimates for each arm by the associated variance derived using the delta-method. (Klein, et al. 2007) [**2**]

8.3.2. Study Objective 3

Study Objective 3 is to evaluate the impact of HCQ on development of IA, that may or may not meet criteria for RA, in high-risk participants 12 months after initiation of study treatment. The clinical endpoint to evaluate this objective will be the development of inflammatory arthritis (IA) at 12 months.

IA is defined as the development of swollen joint(s) consistent with RA-like synovitis. The joint exams conducted at each clinic visit will be used to identify the presence of swollen joints due to IA.

Table 10: Estimand of Study Objective 3

Variable: Time to development of IA	
Estimand Label: Secondary 3.1	
Population: mITT	**IES Policy 1**
Population Level Summary: Estimated risk at 12 months for each treatment arm and the difference between the treatment arms (with 95% confidence intervals) will be derived from Kaplan-Meier (KM) curves and using the Greenwood formula for estimating the variance.	**Analysis**: The analysis will be based on the KM estimated risk of IA at 12 months, where risk = (1-KM estimated probability of "survival"). Survival for this analysis is defined as absence of IA. Estimated risks will be derived from a Kaplan-Meier curve using censored time-to-event data to account for attrition under the assumption of non-informative censoring. The test statistic will be a Wald-type chi-square statistic derived by dividing the difference of the logit-transformed KM survival estimates for each arm by the associated variance derived using the delta-method. (Klein, et al. 2007) [**2**]

8.3.3. Study Objective 4

Study Objective 4 is to evaluate the impact of a 12-month course of HCQ on the timing of development of CL-RA over the entire study period. The clinical endpoint to evaluate this objective will be the development of CL-RA through 36 months. Details on the derivation of the development of CL-RA are provided in section 8.2.1.

Table 11: Estimands of Study Objective 4

Variable: Time to development of CL-RA	
Estimand Label: Secondary 4.1	
Population: mITT	**IES Policy 1**
Population Level Summary: Kaplan-Meier (KM) survival curves (with 95% confidence intervals) will be estimated from baseline through Month 36 for each treatment arm.	**Analysis:** The analysis will be based on the KM survival curves for time to CL-RA from baseline through 36 months. Survival for this analysis is defined as absence of CL-RA. Time-to-event data are censored under the assumption of non-informative censoring. The log rank test will be used to compare HCQ vs. placebo.
Estimand Label: Secondary 4.2	
Population: mITT	**IES Policy 1**
Population Level Summary: Estimated hazard ratios (and 95% confidence intervals) associated with the treatment effect derived from the Cox proportional hazards model.	**Analysis**: A Cox proportional hazards model will be fit with covariates for site (Colorado vs. other sites) and method of recruitment, along with treatment interactions. Only interaction terms with p-values<0.05 will be retained in the model. Wald chi-square tests will be used generate p-values associated with HRs for the treatment effect. Note: The validity of the proportional hazards assumption will be evaluated.
Note: Originally, the method of Lee, Wei, and Amato (Lee, et al. 1992) [**3**] was planned to estimate HRs for the treatment effect from the marginal Cox models for clustered data under assumptions of independent and exchangeable correlation structures to account for familiar clustering. However, familial cluster was not an issue for this study, so only the traditional Cox proportional hazards model will be performed.	
Estimand Label: Secondary 4.3	
Population: mITT	**IES Policy 1**
Population Level Summary: Kaplan-Meier (KM) survival curves (with 95% confidence intervals) will be estimated from baseline through Month 36 for each group defined by site (Colorado vs other) and treatment arm.	**Analysis:** The analysis will be based on the KM survival curves for time to CL-RA from baseline through 36 months. Survival for this analysis is defined as absence of CL-RA. Time-to-event data are censored under the assumption of non-informative censoring. Log rank tests will be used to compare HCQ vs. placebo, and to compare the Colorado study site vs. all other study sites within each treatment arm.

8.3.4. Study Objective 5

Study Objective 5 is to evaluate the impact of a 12-month course of HCQ on the timing of development of IA, that may or may not meet criteria for RA, over the entire study period. The clinical endpoint to evaluate this objective will be the development of inflammatory arthritis (IA) through 36 months.

IA is defined as the development of swollen joint(s) consistent with RA-like synovitis. The joint exams conducted at each clinic visit will be used to identify the presence of swollen joints due to IA.

Table 12: Estimands of Study Objective 5

Variable: Time to development of IA	
Estimand Label: Secondary 5.1	
Population: mITT	**IES Policy 1**
Population Level Summary: Kaplan-Meier (KM) survival curves (with 95% confidence intervals) will be estimated from baseline through Month 36 for each treatment arm.	**Analysis:** The analysis will be based on the KM survival curves for time to IA from baseline through 36 months. Survival for this analysis is defined as absence of IA. Time-to-event data are censored under the assumption of non-informative censoring. The log rank test will be used to compare HCQ vs. placebo.
Estimand Label: Secondary 5.2	
Population: mITT	**IES Policy 1**
Population Level Summary: Estimated hazard ratios (and 95% confidence intervals) associated with the treatment effect derived from the Cox proportional hazards model.	**Analysis**: A Cox proportional hazards model will be fit with covariates for site (Colorado vs. other sites) and method of recruitment, along with treatment interactions. Only interaction terms with p-values<0.05 will be retained in the model. Wald chi-square tests will be used generate p-values associated with HRs for the treatment effect. Note: The validity of the proportional hazards assumption will be evaluated.
NOTE: Originally, the method of Lee, Wei, and Amato (Lee, et al. 1992) [**3**] was planned to estimate HRs for the treatment effect from the marginal Cox models for clustered data under assumptions of independent and exchangeable correlation structures to account for familiar clustering. However, familial cluster was not an issue for this study, so only the traditional Cox proportional hazards model will be performed.	
Estimand Label: Secondary 5.3	
Population: mITT	**IES Policy 1**
Population Level Summary: Kaplan-Meier (KM) survival curves (with 95% confidence intervals) will be estimated from baseline through Month 36 for each group defined by site (Colorado vs other) and treatment arm.	**Analysis:** The analysis will be based on the KM survival curves for time to IA from baseline through 36 months. Survival for this analysis is defined as absence of IA. Time-to-event data are censored under the assumption of non-informative censoring. Log rank tests will be used to compare HCQ vs. placebo, and to compare the Colorado study site vs. all other study sites within each treatment arm.

8.3.5. Study Objective 6

Study Objective 6 is to explore the relationship between baseline and evolving symptoms, risk factors and the development of future CL- RA and response to HCQ. Baseline RA symptoms include self-reported joint pain, stiffness, and swelling, and overall fatigue. Risk factors include but are not limited to age, sex, genetic factors, socio-economic status, education, tobacco exposure, medications and medical hormone use, and dietary factors. The clinical endpoint to evaluate this objective will be the development of CL-RA through 36 months. Details on the derivation of the development of CL-RA are provided in section 8.2.1.

Table 13: Estimand of Study Objective 6

Variable: Time to development of CL-RA	
Estimand Label: Secondary/Exploratory 6.1	
Population: mITT	**IES Policy 1**
Population Level Summary: Hazard ratios (HRs) associated with potential risk factors and modifiers to the HR for the treatment effect comparing HCQ vs. placebo will be estimated with 95% CIs based on a Cox proportional hazards model.	**Analysis**: In the first step in the analysis, fixed effect characteristics of participants who developed CL-RA will be compared to those who did not develop CL-RA during the study using descriptive (e.g., means (SD) or n (%)) and inferential statistics (e.g., Mann-Whitney or Fisher-type exact statistics) appropriate for the nature of the variable. In the next step, the impact of potential risk factors on the development of RA and modification of the treatment effect will be explored using a Cox proportional hazards model. To identify a subset of risk factors for possible inclusion in a final model, we will first run preliminary models each with one potential risk factor, treatment, and the treatment*risk factor interaction. Risk factors with p-values <0.1, and interaction terms with p-values <0.05 will be considered for the final model. The same criteria for inclusion will be used for the final model. Interaction terms will be evaluated first, dropping least significant terms one-by-one until only interactions with p<0.05 remain. Fixed effect components of interaction terms will be included in the final model regardless of p-value. For other fixed effect terms, least significant terms will be dropped one-by-one until only terms with p<0.1 remain. Note: The validity of the proportional hazards assumption will be evaluated. Finally, if results of analyses described in Section 8.3.6, Study Objective 7 or Section 8.3.7, Study Objective 8 suggest that evolving symptoms or risk factors are potentially related to the development of CL-RA, we will consider adding time-varying covariates to the model developed above. The modeling approach will follow that outlined above. Time-varying covariates must be carefully defined prior to modeling, and models must be interpreted with caution. Note: The time-varying covariate model will be exploratory and only undertaken if warranted by results of other analyses.

Fixed effects (i.e., potential risk factors) to be evaluated include:

- Study site (Colorado vs other sites)
- Method of recruitment (FDR, Population-based screening, rheumatology clinic)
- Baseline self-reported joint pain
- Baseline self-reported joint stiffness
- Baseline self-reported joint swelling
- Baseline self-reported fatigue
- Baseline CDAI
- Baseline SDAI
- Baseline RAPID-3
- Baseline DAS28-CRP

- Baseline PROMIS Profile 29 Physical Health Score
- Baseline PROMIS Profile 29 Mental Health Score
- Baseline PROMIS Profile 29 Social Health Score
- Baseline Anti-CCP3 (units)
- Baseline Anti-CCP3 Positivity (Negative (< Upper Limit of Normal (ULN), Low Positive (1.0 - <2.0 x ULN), Medium Positive (2.0 - <3.0 x ULN), High Positive (≥3.0 x ULN))
- Baseline IgM-RF (U/mL)
- Baseline IgM-RF Positivity (Negative (< Upper Limit of Normal (ULN), Low Positive (1.0 - <2.0 x ULN), Medium Positive (2.0 - <3.0 x ULN), High Positive (≥3.0 x ULN))
- Baseline hs-CRP (mg/L)
- Baseline hs-CRP Positivity (≤ 3.0 mg/L, > 3.0 mg/L)
- Baseline age (years)
- Sex (male/female)
- BMI
- Baseline Shared Epitope (SE) Positivity) (Yes, No)
- Baseline Shared Epitope Number of Positive Results (0, 1, 2)
- Participant has a FDR with RA (Yes, No)
- Salary level (< $50,00; $50,000 - < $75,000; ≥$75,000)
- Highest education level achieved (up to High School/GED, College, Graduate School)
- Tobacco exposure
    - Smoking status (Never smoked, Former smoker, Current smoker)
    - Baseline Pack Years at Current Level for Smokers
    - Years as Adult Living with Smoker
- Baseline Medications
    - Statin use (Yes/No)
    - Medical hormones (Yes/No)

Each fixed effect will be evaluated for its impact on the time to development of CL-RA by fitting a Cox proportional hazards model with independent variables for treatment arm, risk factor and treatment arm * risk factor interaction. The risk factors with significant p-values for the Wald chi-square test statistic, along with significant interaction terms, will be included in the final Cox proportional hazards model.

8.3.6. Study Objective 7

Study Objective 7 is to evaluate the relationship between treatment with HCQ and amelioration of symptoms of RA, and potential delay in onset of symptoms. RA symptoms include self-reported joint pain, stiffness, and swelling, and overall fatigue. The clinical endpoint to evaluate this objective will be the self-reported joint symptom counts assessed at each clinic visit.

Participants use the Participant Self-Reported Joint Symptoms assessment to identify painful, stiff, and swollen joints. These data are used to count the number of (1) painful, (2) stiff, and (3) swollen joints, as well as the count of (4) painful, (5) stiff, and (6) swollen joints in the hands, wrists, and feet.

Table 14: Estimands of Study Objective 7

<table>
<tr><th>Estimand Label</th><th colspan="2">Variable</th></tr>
<tr><td>Secondary 7.1</td><td colspan="2">Number of self-reported painful joints at each clinic visit</td></tr>
<tr><td>Secondary 7.2</td><td colspan="2">Number of self-reported stiff joints at each clinic visit.</td></tr>
<tr><td>Secondary 7.3</td><td colspan="2">Number of self-reported swollen joints at each clinic visit</td></tr>
<tr><td>Secondary 7.4</td><td colspan="2">Number of self-reported painful joints in the hands, wrists, and feet at each clinic visit</td></tr>
<tr><td>Secondary 7.5</td><td colspan="2">Number of self-reported stiff joints in the hands, wrists, and feet at each clinic visit</td></tr>
<tr><td>Secondary 7.6</td><td colspan="2">Number of self-reported swollen joints in the hands, wrists, and feet at each clinic visit</td></tr>
<tr><td>Population: mITT</td><td colspan="2">IES Policy: All available study data will be included.</td></tr>
<tr><td>Population Level Summary:
At each planned clinic visit, the number, and percent of individuals with self-reported joint symptoms will be computed by treatment arm, and separately for those with and without a prior study-confirmed diagnosis of CL- RA. Among those with symptoms, mean, median, min and max # of affected joints will also be reported. Summary statistics will also be plotted over time to gain a better understanding of trends.</td><td colspan="2">Analysis: At Week 52 and Month 36/EOS, Mann-Whitney U statistics will be used to compare joint counts between treatment arms, separately for those with and without a diagnosis of CL-RA.

Because the distributional characteristics of these variables are unknown, we cannot prespecify the planned analysis strategy. However, in this population, it is reasonable to expect there will be excess # of participant visits where no joint symptoms are reported. If this is the case, it may appropriate to fit a longitudinal zero-inflated negative binomial model with the number of involved joints as the dependent variable and treatment arm as the primary fixed effect. Important additional fixed effects to consider would include prior CL-RA diagnosis at time of assessment (Y/N), study day (or visit), treatment period (on study drug vs off study drug), duration on study treatment, and interaction terms for treatment arm, prior CL-RA diagnosis, and study day (or visit). Heterogeneous random participant effects could be included in the model per the methods of Zhu et al [4], and relevant covariates included in the regression on the covariance matrix will be determined. T-tests for the maximum likelihood estimates of the model parameters could be used to determine the significance of the parameters to the model.</td></tr>
<tr><td colspan="3">Note: Modeling will be exploratory and only initiated if descriptive analyses suggest interesting trends.</td></tr>
</table>

8.3.7. Study Objective 8

Study Objective 8 is to explore underlying immune responses over time in the early natural history of RA development and in response to HCQ therapy through measurement of a variety of biomarkers. Endpoints will be laboratory test results from mechanistic specimens collected at specified clinic visits during the study.

Table 15: Estimands of Study Objective 8

<table>
<tr><th>Estimand Label</th><th colspan="2">Biomarkers and immune response variables</th></tr>
<tr><td>Secondary 8.1</td><td colspan="2">Levels of anti-CCP3 over time</td></tr>
<tr><td>Secondary 8.2</td><td colspan="2">Levels of IgM-RF over time</td></tr>
<tr><td>Secondary 8.3</td><td colspan="2">Levels of hsCRP over time</td></tr>
<tr><td>Population: mITT</td><td colspan="2">IES Policy: All available study data will be included.</td></tr>
<tr><td colspan="2">Population Level Summary: Summary statistics and plots will be used to gain an understanding of the data prior to developing any statistical models or hypothesis tests.
For categorical variables, frequencies and percents will be computed at each time point by treatment group and separately by participants who were or were not diagnosed with CL-RA.
For continuous variable, the number, mean (SD), median, min, and max score will be computed at each planned clinic visit, by treatment arm, and separately for those with and without a prior study-confirmed diagnosis of CL-RA. If any continuous variables that have values below the limit of detection, dichotomous variable will be created and summarize.
Summary statistics will also be plotted over time to gain a better understanding of trends.</td><td>Analysis: Inferential analyses will be considered if descriptive analyses suggest interesting trends.</td></tr>
<tr><td colspan="3">Note: All mechanistic data is generated by core laboratory facilities, which may also run analyses.</td></tr>
</table>

8.4. Exploratory Endpoints

Exploratory endpoints that are not directly related to a specific study objective are included with the planned estimands.

8.4.1. Physician-reported Joint Counts

Physicians use the Physician Joint Exam at every clinic visit to identify tender and swollen joints. These data are used to count the number of (1) tender and (2) swollen joints.

Table 16: Estimands of Physician-reported Joint Counts

<table>
<tr><th>Estimate Label</th><th colspan="2">Count Variables</th></tr>
<tr><td>Secondary 9.1</td><td colspan="2">Number of physician-identified tender joints at each clinic visit</td></tr>
<tr><td>Secondary 9.2</td><td colspan="2">Number of physician-identified swollen joints at each clinic visit</td></tr>
<tr><td>Population: mITT</td><td colspan="2">IES Policy: All available study data will be included.</td></tr>
<tr><td colspan="2">Population Level Summary: Population Level Summary:
At each planned clinic visit, the number, and percent of individuals with physician-identified tender joints (or swollen joints) will be computed by treatment arm, and separately for those with and without a prior study-confirmed diagnosis of CL- RA. Among those with tender (or swollen joints), mean, median, min and max # of affected joints will also be reported. Summary statistics will also be plotted over time to gain a better understanding of trends.</td><td>Analysis: At Week 52 and Month 36/EOS, Mann-Whitney U statistics will be used to compare joint counts between treatment arms, separately for those with and without a diagnosis of CL-RA.

Because the distributional characteristics these variables are unknown, we cannot prespecify the planned analysis strategy. However, in this population, it is reasonable to expect there will be excess # of participant visits where no tender joints (or swollen joints) are identified. If this is the case, it may appropriate to fit a longitudinal zero-inflated negative binomial model with the number of involved joints as the dependent variable and treatment arm as the primary fixed effect. Important additional fixed effects to consider would include prior CL-RA diagnosis at time of assessment (Y/N), study day (or visit), treatment period (on study drug vs off study drug), duration on study treatment, and interaction terms for treatment arm, prior CL-RA diagnosis, and study day (or visit). Heterogeneous random participant effects could be included in the model per the methods of Zhu et al [4], and relevant covariates included in the regression on the covariance matrix will be determined. T-tests for the maximum likelihood estimates of the model parameters could be used to determine the significance of the parameters to the model.</td></tr>
<tr><td colspan="3">Note: Modeling will be exploratory and only initiated if descriptive analyses suggest interesting trends.</td></tr>
</table>

8.4.2. Disease Activity Assessments

The DAS28-CRP score is calculated from the tender and swollen joint counts from the Physicians Joint Exam, the laboratory result for hsCRP and the Overall Health score from the MDHAQ. The score will be calculated at the visits where the real-time core outcome hsCRP sample was collected.

The CDAI score is calculated from the tender and swollen joint evaluations in the Physicians Joint Exam, the Overall Health score from the MDHAQ assessment and the Physicians Global Assessment of Overall Disease Activity in the Physicians Assessment.

The SDAI score is calculated from the tender and swollen joint evaluations in the Physicians Joint Exam, the Overall Health score from the MDHAQ assessment and the Physicians Global Assessment of Overall Disease Activity in the Physicians Assessment, and the hsCRP level.

The RAPID-3 score is calculated from the MDHAQ assessment.

NIH PROMIS Profile 29 is a patient reported outcome (PRO) assessment with multiple modules that are used to calculate the Physical Health score, Mental Health score, and Social Health score. The score will be calculated at the visits when the PROMIS Profile 29 assessments were conducted. The PROMIS Profile 29 scores will be calculated using a validated scoring method (https://www.healthmeasures.net/index.php?option=com_content&view=category&layout=blog&id=190&Itemid=1214)

Table 17: Estimands of Disease Activity Assessments

<table>
<tr><th>Estimate Label</th><th colspan="2">Scale Score Variables</th></tr>
<tr><td>Secondary 9.3</td><td colspan="2">DAS28-CRP score at each clinic visit.</td></tr>
<tr><td>Secondary 9.4</td><td colspan="2">CDAI at each clinic visit.</td></tr>
<tr><td>Secondary 9.5</td><td colspan="2">RAPID-3 score at each clinic visit.</td></tr>
<tr><td>Secondary 9.5</td><td colspan="2">PROMIS Profile 29 Physical health score at each clinic visit.</td></tr>
<tr><td>Secondary 9.6</td><td colspan="2">PROMIS Profile 29 Mental health score at each clinic visit.</td></tr>
<tr><td>Secondary 9.7</td><td colspan="2">PROMIS Profile 29 Social health score at each clinic visit</td></tr>
<tr><td>Population: mITT</td><td colspan="2">IES Policy: All available study data will be included.</td></tr>
<tr><td colspan="2">Population Level Summary:
At each planned clinic visit, the number, mean (SD), median, min, and max score will be computed by treatment arm, and separately for those with and without a prior study-confirmed diagnosis of CL- RA. Summary statistics will also be plotted over time to gain a better understanding of trends.</td><td>Analysis: At Week 52 and Month 36/EOS, Mann-Whitney U statistics will be used to compare scores between treatment arms, separately for those with and without a diagnosis of CL-RA.

If data are consistent with a Gaussian distribution, the data may be analyzed using a repeated measure mixed regression model with the scale score variable at each visit as the dependent variable and treatment arms as the primary fixed effect. Important additional fixed effects to consider would include prior CL-RA diagnosis at time of assessment (Y/N), study day (or visit), treatment period (on study drug vs off study drug), duration on study treatment, and interaction terms for treatment arm, prior CL-RA diagnosis, and study day (or visit). Random participant effects may include random intercepts and/or slopes for each participant</td></tr>
<tr><td colspan="3">Note: Modeling will be exploratory and only initiated if descriptive analyses suggest interesting trends.</td></tr>
</table>

9. SAFETY EVALUATIONS

9.1. Overview of Safety Analysis Methods

All safety analyses will be carried out using the Safety Population defined in Section 5.1 unless otherwise noted. Missing safety information will not be imputed. These analyses will not be stratified by site.

Safety will be analyzed in both treatment arms through the reporting of adverse events (AEs), vital signs, physical examination findings, and changes in routine laboratory values.

Listings will be prepared for all safety measurements. All listings will be sorted in order of treatment, participant identifier (ID), and time of assessment (e.g., visit, time, and/or event).

9.2. Extent of Exposure

Duration of exposure will be defined as the last dose date – first dose date + 1. Descriptive statistics will be presented by treatment arm.

9.3. Adverse Events

All AEs will be classified by system organ class (SOC) and preferred term, according to a standardized thesaurus (Medical Dictionary for Regulatory Activities [MedDRA] version 23.0). The severity of AEs will be classified using the National Cancer Institute's (NCI's) Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0. Each AE is entered on the electronic case report form (CRF) once at the highest severity. As such, no additional data manipulation is needed to identify events.

AEs will be collected starting at consent for Grade 2 or higher AEs related to a study-mandated procedure, treatment, or change in treatment, and will be collected starting from the first dose of study treatment through Month 18 or until 30 days after the participant prematurely terminates from the study, whichever occurs first. SAEs are collected for participants throughout the duration of their participation in the study. Treatment-emergent AEs will be identified as those with an onset date on or after the first dose of study medication. If the start of the AE in relation to the start of study medication cannot be established (e.g., the start date for the AE is missing), then the AE will be considered treatment-emergent. If an abnormal laboratory finding is reported as an AE on the first day of study drug (Day 1), these events will not be considered treatment emergent since these assessments occur prior to treatment initiation. All data tabulations will be of only treatment-emergent events while non-treatment-emergent AEs will be included in the listing of all AEs.

The frequency of AEs will be summarized by system organ class, preferred term, severity (grade), and relationship to study treatment. Relationship to study treatment, as determined by masked investigators, will be categorized as either treatment related (possibly, probably, or definitely related to study medication) or unrelated (unlikely related or unrelated). Similar analyses will be performed for SAEs. To account for differential duration of study participation among participants, the summaries will also include the event rate (i.e., number of events per person-time) in addition to the number and percent of events and participants experiencing events.

For the secondary safety endpoint of the proportion of participants in each arm experiencing a Grade 3 or higher AE according to the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) system, the proportion of participants experiencing at least one event in each treatment group will be reported and the treatment groups compared based on Fisher's Exact Test.

9.4. Deaths, Serious Adverse Events, and Other Significant Adverse Events

The frequency of SAEs will be summarized by system organ class, preferred term, severity (grade), and relationship to study treatment. Relationship to study treatment, as determined by masked investigators, will be categorized as either treatment related (possibly, probably, or definitely related to study medication) or unrelated (unlikely related or unrelated). To account for differential duration of study participation among participants, the summaries will also include the event rate (i.e., number of events per person-time) in addition to the number and percent of events and participants experiencing events.

9.5. Clinical Laboratory Evaluation

For these analyses, clinical laboratory measurements include serum chemistry and hematology, and exclude autoantibody and CRP levels, which will be evaluated in the Secondary Objectives. Laboratory results will be reported from a central lab. However, sites can additionally report unscheduled results performed locally in the EDC. Local results will be converted to standardized units where possible. Changes in laboratory parameters that represent increases NCI-CTCAE severity grade over time are captured as AEs and summarized as described in Section 9.3.

Laboratory parameters will be summarized both overall and by treatment group using appropriate descriptive statistics. For each lab parameter, the number and percent of participants that have an increase, decrease, or no change from Baseline to Week 24 and Week 52 will be displayed for each treatment group and pooled across treatment arms. For parameters with an explicit NCI-CTCAE grading criterion, change from baseline will be indicated by a change in grade. For parameters that do not have an explicit NCI-CTCAE grading criterion, observed values will be categorized as 'high' (defined as >ULN), 'normal' (defined as ≥ lower limit of normal (LLN) and ≤ ULN), or 'low' (defined as <LLN). Then, a change from baseline will be indicated as a change in category.

Laboratory data will also be plotted to show patterns over time. Summary statistics including 25th percentile, median, and 75th percentile will be plotted for each visit by treatment group. Lines connecting individual participant results from participants with Grade 2 or higher values will be overlaid on each figure. For lab results that are not gradable, results from participants with values outside of 2 *ULN or 0.5*LLN will be overlaid.

10. OTHER ANALYSES

Other analyses that may be considered post-hoc include:

- For participants who developed CL-RA during the study, we may examine characteristics and features of the diagnosis by treatment group. The features of diagnosis may include (but are not limited to) disease activity measures (e.g. DAS28CP), x-ray findings (e.g. presence of erosions and Sharp's scores) and persistence of tender and swollen joints post-diagnosis.
- For participants who developed CL-RA during the study, we may examine the severity of the CL-RA symptoms at the time of diagnosis by treatment group. Severity can be measured using DAS28-CRP, CDAI, RAPID-3, and PROMIS scores at the time of diagnosis.
- We may examine relationships between dietary intake and/or other environmental exposures and development of the CL-RA.

Additional mechanistic analyses may be performed, and the details of those analyses will be specified in separate statistical analysis plans.

11. INTERIM ANALYSES AND DATA MONITORING

11.1. Interim Analysis of the Primary Objective

The details for the planned interim analyses are provided in the ARA08 Interim Statistical Analysis Plan, which was finalized as version 1.0 on December 17, 2021. The ARA08 Interim Statistical Analysis Plan is included as Appendix 14.1.

The first interim analysis was conducted in April 2022, and the results of the interim analysis, "Interim Analysis for the April 2022 DSMB", were included as an appendix in the closed report to the Data and Safety Monitoring Board on April 6, 2022 as part of the regularly scheduled review of the ARA08 study.

12. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

- Protocol section 8.3.1.2 states "All efficacy analyses will be repeated using the PP population." We have modified this plan for secondary efficacy analyses. PP analyses maybe conducted at the discretion of the study team if warranted by results of the mITT analyses.
- Protocol section 8.3.1.1 states “The primary analysis ignores the possible impact of within-family correlation. The propensity to progress to RA could be more similar within families than between families or across individuals in the population, in which case observations in this study would not be independent. Due to operational difficulties in linking family members, we expect to have incomplete information on familial clustering, so we will not be able to account for this potential correlation in the primary analysis. Because families tend to be small and not all family members will be eligible or willing to participate, we anticipate the impact of clustering to be small. We will, however, perform sensitivity analyses to assess the potential impact of clustering. We are asking participants if they have FDRs who are participating. If the proportion who answer affirmative is small, the impact of clustering is likely to be minimal. In addition, participants have the option of linking their study records with those of their relatives. If family clusters are identified through this process, we can estimate the treatment effect from a marginal Cox model for clustered data using the method of Lee, Wei, and Amato [178] and compare these to estimates derived from the usual Cox model assuming independent observations. We could also compare estimates for the risk of developing RA derived from logistic regression models fit using generalized estimating equations under difference assumptions about the within-family correlation structure; independent versus exchangeable [179].”

  Upon review of the data collected in ARA08 regarding members of the same family participating in ARA08, it was discovered that no participants reported having an FDR who was also participating in the study. As a result, sensitivity analyses to assess the impact of family clustering will not be performed.

13. REFERENCES

1. Pocock, S.J. and R. Simon, Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics, 1975. 31(1): p. 103-15.
2. Klein, J.P., et al., Analyzing survival curves at a fixed point in time. Stat Med, 2007. 26(24): p. 4505-19.
3. Lee, E.W., et al., Cox-Type Regression Analysis for Large Numbers of Small Groups of Correlated Failure Time Observations, in Survival Analysis: State of the Art, J.P. Klein and P.K. Goel, Editors. 1992, Springer Netherlands: Dordrecht. p. 237-247.
4. Zhu, H., et al., Zero-inflated count models for longitudinal measurements with heterogeneous random effects. Stat Methods Med Res. 2017 August; 26(4): 1774–1786

14. APPENDIX

14.1. Interim Statistical Analysis Plan

INTERIM STATISTICAL ANALYSIS PLAN

STUDY TITLE:

Strategy to Prevent the Onset of Clinically-Apparent Rheumatoid Arthritis (StopRA)

PROTOCOL NUMBER:

ARA08

SHORT TITLE:	StopRA
NCT#:	NCT02603146
COMPOUND:	Hydroxychloroquine
SPONSOR:	Division of Allergy, Immunology, and Transplantation National Institute of Allergy and Infectious Diseases – NIH
	[redacted]
REGULATORY AGENCY IDENTIFIER NUMBER(S):	Non-IND
PREPARED BY:	[redacted]

This document is confidential and proprietary to DAIT/NIAID. This study is being conducted in compliance with good clinical practice, including the archiving of essential documents. Acceptance of this document constitutes agreement by the recipient that no unpublished information contained herein will be reproduced, published, or otherwise disclosed without the prior written approval of DAIT/NIAID, except that this document may be disclosed to appropriate Institutional Review Boards under the condition that they keep the information confidential.

ACKNOWLEDGEMENT AND SIGNATURE SHEET

Approved:	**Approved:**
Kevin Deane, MD, PhD Protocol Chair	[redacted] NIH/NIAID/DAIT Medical Monitor
Signature and Date	Signature and Date
DocuSigned by:  Signer Name: Kevin Deane Signing Reason: I have reviewed this document Signing Time: 21-Dec-2021 | 4:50:23 PM EST 0CBDBD077DAF4126AC2597245B3BD928	DocuSigned by: [redacted]

Approved:	**Approved:**
[redacted] Senior Statistician	[redacted] Senior Statistical Reviewer
Signature and Date	Signature and Date
DocuSigned by: [redacted]	DocuSigned by: [redacted]

VERSION HISTORY

SAP Version	Version Date	Change(s)	Rationale
1.0	17Dec2021		Final Version

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Table 1: List of Abbreviations

Abbreviation	Term
ACPAs	Antibodies to Citrullinated Protein Antigens
Anti-CCP3	Anti-cyclic citrullinated peptide-3
CRF	Case Report Form
CSR	Clinical Study Report
FDR	First-degree relative
HCQ	Hydroxychloroquine
IA	Inflammatory arthritis
ISAP	Interim Statistical Analysis Plan
IBW	Ideal body weight
ITT	Intent-to-Treat Population
KM	Kaplan-Meier
Max	Maximum
Min	Minimum
mITT	Modified Intent-to-Treat Population
PP	Per Protocol Population
RA	Rheumatoid Arthritis
SAP	Statistical Analysis Plan
SD	Standard deviation
SP	Safety Population

1. PURPOSE OF THE ANALYSES

We will conduct one or possibly two interim analyses to stop the study early for overwhelming evidence of efficacy or futility. Results from the first interim analyses will supplement the usual safety and study conduct information presented to the Data and Safety Monitoring Board (DSMB) at the spring 2022 meeting. Results of these analyses are not binding. The DSMB will provide recommendations on study continuation to DAIT. In the event of a recommendation to stop the study, DAIT will convene an unmasked panel to consider the recommendations.

The purpose of this interim statistical analysis plan (ISAP) is to describe the planned interim efficacy and futility analyses and associated data displays to be included in the interim analysis reports for Protocol ARA08. This document provides details on the analysis population, derivation of variables, plans for handling of missing data, and statistical methods to be used in these analyses.

This ISAP is based on ICH guidelines E3 and E9 (Statistical Principles for Clinical Trials).

2. PROTOCOL SUMMARY

Title of the Protocol: Strategy to Prevent the Onset of Clinically-Apparent Rheumatoid Arthritis (StopRA)

ACE Protocol Number: ARA08

Protocol Chair(s): Dr. Kevin Deane, MD, PhD

Sponsor: DAIT/NIAID/NIH

Objectives:
The primary objective is to determine the efficacy of a 12-month course of hydroxychloroquine (HCQ) to prevent the development of clinically-apparent rheumatoid arthritis (RA) (as defined in Section 2.1, *Primary Objective)* at 36 months in participants at high-risk for future RA due to high titer elevations of anti-cyclic citrullinated peptide-3 (anti-CCP3) (≥ 40 units) but who are without a history or clinical findings of inflammatory arthritis (IA) at Baseline.

Secondary objectives include:

1. To evaluate the safety of a 12-month course of HCQ in participants who are at high-risk for development of RA.
2. To evaluate the impact of HCQ on development of clinically-apparent RA (as defined in Section 2.1, *Primary Objective*) in high-risk participants 12 months after initiation of study treatment.
3. To evaluate the impact of HCQ on development of IA that may or may not meet criteria for RA in high-risk participants 12 months after initiation of study treatment.
4. To evaluate the impact of a 12-month course of HCQ on the timing of development of clinically-apparent RA over the entire study period.
5. To evaluate the impact of a 12-month course of HCQ on the timing of development of IA, that may or may not meet criteria for RA, over the entire study period.
6. To explore the relationship between baseline and evolving symptoms[1], risk factors[2] and the development of future clinically-apparent RA and response to HCQ.
7. To evaluate the relationship between treatment with HCQ and amelioration of symptoms [1] of RA, and potential delay in onset of symptoms.
8. To explore underlying immune responses over time in the early natural history of RA development and in response to HCQ therapy through measurement of a variety of biomarkers.

Study Arms:

- Hydroxychloroquine: These participants will receive 200 - 400 mg of HCQ (1-2 pills), based upon ideal body weight (IBW) at Screening, daily for 12 months.
- Placebo: These participants will receive 1-2 pills of placebo (based upon IBW at Screening) daily for 12 months.

Study Design: This is a phase 2 multi-center, randomized, placebo-controlled, double-blind, parallel group 36-month clinical trial to evaluate the effectiveness and safety of intervention with a 12-month course of HCQ to prevent the future onset of clinically-apparent RA (See definition in Section 2.1, *Primary Objective*). At screening, study participants will be without IA, but will be at high-risk for developing future RA within the trial period as indicated by elevated anti-CCP3 antibodies that are ≥40 units (that is a level ≥2 times the normal cut-off of ≥20 units). Two-hundred eligible participants will be randomized in a 1:1 ratio to receive either self-administered HCQ or placebo.

Participants will provide informed consent prior to any Pre-Screening or screening procedures. Participants who are found to be eligible after the screening evaluation will return for a Baseline/Randomization visit within 30 days of the initial screening visit. Participant eligibility will be confirmed prior to randomization.

[1] Baseline RA symptoms include self-reported joint pain, stiffness, and swelling, and overall fatigue.
[2] Risk factors include but are not limited to age, sex, genetic factors, socio-economic status, education, tobacco exposure, medications and medical hormone use, and dietary factors.

Title of the Protocol: Strategy to Prevent the Onset of Clinically-Apparent Rheumatoid Arthritis (StopRA)
Eligible participants will be randomized to receive either 200 - 400 mg (1-2 pills) of HCQ or 1-2 pills of placebo daily for 12 months based upon IBW at Screening. The weight-based dosing regimen for the study is outlined in Section 5.2, *Dosage Regimen*. Participants will return to the study site for planned evaluations at Week 6, 12, 24, 36, and 52 (End of Treatment), and Months 18, 24, 30, and 36 (End of Study). During these study visits, participants will have a joint exam and a physical examination. Study personnel will record the participant's interval medical history, assess adverse events, and collect samples for safety and mechanistic assessments (see Tables 6.1 & 6.2, *Schedule of Events*). Information on demographics (including socio-economic status and education), and other factors that may influence autoimmunity (e.g. tobacco exposure, hormonal status and exposures) may also be collected. Site coordinators will call participants at Week 18, 30, and 42 and at Month 15, 21, 27, and 33 to answer participant questions, update contact information, and to assess AEs/reactions, study drug dosing and pregnancy status (during the treatment period), and joint symptoms. If a participant indicates that he/she is experiencing joint symptoms suggestive of RA (that include new or worsening joint pain, stiffness or swelling since the prior study visit), or symptoms suggestive of an AE, the participant will be asked to return to the study site as soon as possible. Visits and assessments for participants who develop RA, IA with erosions, or who become pregnant prior to the Month 36 visits will be different from participants who never develop RA. Details of these assessments may be found in protocol sections 6.5.9, *Evaluations Triggered by a Swollen Joint,* 6.5.10, *Procedures for Participants Diagnosed with Inflammatory Arthritis or Rheumatoid Arthritis by an Outside Physician,* and 6.5.11, *Special Considerations for Pregnant Participants*.
Endpoints: The primary efficacy endpoint is the development of clinically-apparent RA by 36 months, where clinically-apparent RA is defined in Section 2.1, *Primary Objective*. Secondary efficacy and safety endpoints are described in Sections 3.3.1, *Secondary Efficacy Endpoints,* and 3.3.2, *Secondary Safety Endpoints.*
Sample Size: 200 eligible participants will be randomized in a 1:1 ratio.
Data Analyses: For the primary analysis, we are interested in demonstrating a long-term impact of a 1-year course of HCQ treatment on preventing the development of clinically-apparent RA (defined in Section 2.1, *Primary Objective*) in high-risk participants. As such, rather than comparing full survival curves between treatment arms, the sample size for this study was selected to achieve sufficient power to compare survival curves at a fixed point 3 years after initiating treatment with HCQ. All secondary analyses will be conducted in an exploratory fashion with p-values and confidence intervals presented as descriptive statistics with no adjustments for multiple comparisons. Tests will be two-sided and interval estimates will be generated at the 95% confidence level.

3. GENERAL ANALYSIS AND REPORTING CONVENTIONS

The following is a list of general analysis and reporting conventions to be applied for this study:

- Categorical variables will be summarized using counts (n) and percents (%) and will be presented in the form n (%).
- Moment statistics including mean will be reported at 1 more significant digit than the precision of the original data. The standard deviation will be reported at 2 more significant digits than the precision of the original data. The level of precision may be modified on specific displays based on clinical judgement.
- Order statistics including median, min and max will be reported to the same level of precision as the original observations. If any values are calculated to have more significant digits then the value should be rounded so that it is the same level of precision as the original data.
- Following SAS default rules, the median will be reported as the average of the two middle numbers if the dataset contains an even number of observations.
- Test statistics including t and z test statistics will be reported to two decimal places.
- P-values will be reported to 3 decimal places if greater than 0.001. If less than 0.001 then report '<0.001'. Report p-values and significant levels as 0.05 rather than .05. A p-value can be reported as "1.000" only if it is exactly 1.000 without rounding. A p-value can be reported as "0.000" only if it is exactly 0.000 without rounding.
- In general, listings will be displayed by treatment group and participant and will be sorted in the order that columns are displayed, starting with the first column on the left.
- All analyses will be performed using the SAS System version 9.4.

4. ANALYSIS POPULATION

All interim analyses for efficacy and futility will utilize the modified intent-to-treat population defined for this study, as follows:

Modified Intent-to-Treat Population

The modified Intent-to-Treat (mITT) population will include all randomized participants who receive at least one dose of study drug and meet entry criteria. The primary efficacy analyses will be based on the mITT population. Participants who, for whatever reason, do not complete their assigned therapy will be included in the mITT population in the groups to which they were randomized.

5. ENDPOINT EVALUATION

5.1. Overview of Analysis Methods

5.1.1. Multicenter Studies

Study participants will be recruited from up to 20 study sites. The analyses of survival estimates at fixed points in time will not be stratified for the interim analyses.

5.1.2. Assessment Time Windows

Visit windows for each scheduled visit are provided in Table 2. The Baseline visit must be performed within 30 days of the Screening visit.

Table 2: Visit Target Days and Windows for Scheduled Visits

Visits	Target Day	Visit Type	Window
Baseline	0	Clinic	≤30 days from Screening
Week 6	42	Clinic	±7 days from target
Week 12	84	Clinic	
Week 18	126	Telephone	
Week 24	168	Clinic	
Week 30	210	Telephone	
Week 36	252	Clinic	
Week 42	294	Telephone	
Week 52	364	Clinic	±14 days from target
Month 15	457	Telephone	
Month 18	548	Clinic	
Month 21	639	Telephone	
Month 24	731	Clinic	
Month 27	822	Telephone	
Month 30	913	Clinic	
Month 33	1004	Telephone	
Month 36	1096	Clinic	

Data collected outside of the visit window will not be removed from analyses. Data collected from unscheduled visits and Time of Diagnosis visits will be included in analyses, except for by-visit summaries.

5.2. Primary Estimand

5.2.1. Definition

The primary objective of the study is to determine the efficacy of a 12-month course of HCQ to prevent the development of clinically-apparent RA at 36 months in participants at high-risk for future RA due to high titer elevations of anti-CCP3 (≥ 40 units) but who are without a history or clinical findings of IA at Baseline. For this study, clinically-apparent RA will be defined using the 2010 ACR/EULAR Classification Criteria as either:

(1) A score of ≥ 6 defining "definite RA" or

(2) A joint examination consistent with RA-like synovitis with ≥ 1 erosion identified via x-ray imaging of the hands, wrists, and feet.

The primary hypotheses to be evaluated at interim and end-of-study analyses are:

H0: RA-free survival at 3 years (36 months) is equal across arms;

HA: RA-free survival at 3 years (36 months) is not equal across arms.

Variable:

Time to development of clinically-apparent RA

Population:

The mITT population will be used for these analyses.

Intercurrent Events Strategies (IES):

1. Participants who terminate early from the study prior to a diagnosis of RA will be censored at the time of early termination.
2. Participants who choose to take off-study HCQ on a continuous basis will be censored at the time this therapy began.

   The rationale for this decision is that our ability to evaluate the impact of a short 12-month course of HCQ on development of RA is confounded if individuals choose to take HCQ, provided by physicians outside of the study. Further, data for individuals assigned to 12-months of placebo and then take off-study HCQ could further confound results.
3. Participants who were diagnosed with RA by an outside physician and started prohibited medications will be censored at the time the RA medications were started. Only cases where data collected on-study confirm the diagnosis using the study-defined primary endpoint definition will count as RA/IA cases in the primary analysis.
4. Participants who started prohibited medications during study participation to treat conditions other than RA will be censored as follows:
   - If a participant is taking a prohibited medication other than corticosteroids, then time-in-study will be censored at the start of the first prohibited medication.
   - If a participant is taking corticosteroids, two courses at a dose ≤60 mg/day for ≤21 days are allowed. If either of these conditions is violated, then time-in-study will be censored at the time when the permitted conditions are violated.

The rationale for this decision is that inflammatory arthritis cannot be accurately diagnosed by the study assessments once prohibited medications have been started.

5. Participants who discontinue study-provided HCQ/placebo prematurely or who are not compliant with planned dosing will be included in the analysis and will not be censored at the time of study drug discontinuation or noncompliance.

Population Level Summary:

Estimated risk at 36 months for each treatment arm and the difference between the treatment arms (with 95% confidence intervals) will be derived from Kaplan-Meier (KM) curves and using the Greenwood formula for estimating the variance.

5.2.2. Interim Analysis of the Primary Estimand

This section presents details on the analysis plans for each interim evaluation. Power estimates are presented in Appendix 8.1.

5.2.2.1. Efficacy

The analysis will be based on the KM estimated risk of clinically-apparent RA at 36 months, where risk = (1-KM estimated probability of "survival"). Survival for this analysis is defined as absence of clinically-apparent RA. Estimated risks will be derived from a Kaplan-Meier curve using censored time-to-event data to account for attrition under the assumption of non-informative censoring.

The test statistic will be a Wald-type chi-square statistic derived by dividing the difference of the logit-transformed KM survival estimates for each arm by the associated variance derived using the delta-method. This analysis will ignore stratification by site and method of recruitment, because the unstratified test statistic has been shown to have better test performance (Klein, et. al. 2007)

The test statistic at each interim analysis stage will be calculated as the logit transformed test statistic in Klein et al (X_5^2):

$$X_{5\,k}^2 = \frac{\left[\log\frac{\widehat{S_{Hk}}(t_{36})}{1-\widehat{S_{Hk}}(t_{36})} - \log\frac{\widehat{S_{Pk}}(t_{36})}{1-\widehat{S_{Pk}}(t_{36})}\right]^2}{\frac{\hat{\sigma}_{Hk}^2(t_{36})}{\left[1-\widehat{S_{Hk}}(t_{36})\right]^2} + \frac{\hat{\sigma}_{Pk}^2(t_{36})}{\left[1-\widehat{S_{Pk}}(t_{36})\right]^2}}$$

where:

$X_{5\,k}^2$ = Test statistic at interim analysis stage k, k = 1 to 2

$\widehat{S_{Hk}}(t_{36})$ = KM estimated probability of RA-free survival in the HCQ treatment arm at month 36 for interim analysis stage k

$\widehat{S_{Pk}}(t_{36})$ = KM estimated probability of RA-free survival in the placebo treatment arm at month 36 for interim analysis stage k

$\hat{\sigma}_{Hk}^2(t_{36})$ = Estimated variance of KM estimated probability of RA-free survival in the HCQ treatment arm at month 36 for interim analysis stage k, calculated via Greenwood's formula

$\hat{\sigma}_{Pk}^2(t_{36})$ = Estimated variance of KM estimated probability of RA-free survival in the placebo treatment arm at month 36 for interim analysis stage k, calculated via Greenwood's formula

The criteria at each interim analysis stage k to determine if the primary null hypothesis should be rejected will be determined by O'Brien-Fleming (OBF) boundary values for the standardized Z scores (Z_{OBFk}). PROC SEQDESIGN will be used to calculate the OBF boundary criteria using information levels based on the proportion of the maximum person-time accumulated on the date of data snapshot for the planned DSMB meeting. Enrollment closed on November 1, 2021 with 144 participants randomized to the study. Hence, the maximum person-years for the study is 144 participants * 3 years = 432 person-years. On the date of the data snapshot, we will compute the information fraction as the sum of time accrued from the day of randomization over all individuals divided by 432. Person-time for individuals randomized more than 3 years prior to the date of the data snapshot will be set at 3.0 person-years. In the example below, we are assuming data snapshot dates of 01FEB2022 and 01FEB2023 for the spring 2022 and spring 2023 DSMB meetings, respectively. Based on the study progress of all randomized participants, the information proportions for each interim analysis stage would be as follows:

Table 3: Estimated Information

Interim Analysis Stage (k)	Time Point	Elapsed Study Time (years)	Elapsed Study Time (person-years)	Estimated Information Proportion (I_k)
1	Spring 2022 DSMB [01FEB2022]	5.8	366.5	0.848
2	Spring 2023 DSMB [01FEB2023]	6.8	405.4	0.938
Final	End of Study [01NOV2024]	8.5	432.0	1.000

Note the actual information proportions will vary depending on the information available on the dates of data cuts.

At each interim analysis stage k, the primary hypothesis will be tested as follows:

- If $X^2_{5k} \geq (Z_{OBFk})^2$, the null hypothesis H_0 that the RA-free survival at 3 years (36 months) is equal across arms is rejected, indicating there is strong evidence of a treatment effect.
- If $X^2_{5k} < (Z_{OBFk})^2$, the null hypothesis H_0 that the RA-free survival at 3 years (36 months) is equal across arms is not rejected.

5.2.2.2. Futility

To evaluate futility, we will compute a Z-score test statistic based on the difference of logit transformed survival estimates, calculated as:

$$Z_k = \frac{\log\frac{\widehat{S_{Hk}}(t_{36})}{1-\widehat{S_{Hk}}(t_{36})} - \log\frac{\widehat{S_{Pk}}(t_{36})}{1-\widehat{S_{Pk}}(t_{36})}}{\sqrt{\frac{\hat{\sigma}^2_{Hk}(t_{36})}{\left[1-\widehat{S_{Hk}}(t_{36})\right]^2} + \frac{\hat{\sigma}^2_{Pk}(t_{36})}{\left[1-\widehat{S_{Pk}}(t_{36})\right]^2}}}$$

A Z-score <0.3 would suggest that there is little chance of detecting a significant survival advantage for the HCQ arm. The test for futility will be conducted as follows:

- If $Z_k < 0.3$, the futility test criterion has been met, and there is a small chance of detecting a significant treatment difference in RA-free survival rates at month 36 at the end the of the study.
- If $Z_k \geq 0.3$, the futility test criterion has not been met.

5.2.3. Interim Analysis of the Sensitivity Estimand

The estimand for these sensitivity analyses will be identical to the primary estimand except for the following updates to the Intercurrent Events Strategy for handling cases for individuals who withdrew from the study early or who began treatment for an unconfirmed diagnosis of RA.

Changes to Intercurrent Events Strategies defined in Section 5.2.1:

1. For participants who terminate early from the study prior to a diagnosis of RA and have consented to be contacted via phone, a participant-reported diagnosis of RA that is (or was) treated will be counted in the sensitivity analysis as a case of RA/IA at the time of initiation of the RA/IA medications. For participants without a reported RA/IA diagnosis, data will be censored at the date of last phone contact. For participants who cannot be contacted, data will be censored at the time of early termination.
2. Participants who choose to take off-study HCQ on a continuous basis will not be censored at the time this therapy began.
3. Participants who were diagnosed with RA by an outside physician and started prohibited medications will be included in the analysis as RA cases at the time the prohibited medications were initiated, regardless of whether or not the diagnosis can be confirmed using study-defined criteria and study data.
4. For participants who terminate early from the study prior to a diagnosis of RA and have consented to be contacted via phone, data for a participant who reports use of a prohibited medication to treat conditions other than RA will be censored at the time the prohibited medication was started.

6. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

In protocol version 3.0, we provided initial details on the plans for the interim analyses to be performed for efficacy and futility for ARA08. Below are changes to the protocol details for the interim analyses:

- The assumptions about enrollment expectations, duration of enrollment and timing of interim analyses presented in protocol version 3.0 are inaccurate. Hence, updated power calculations are presented in Appendix 8.1.
- In the protocol, we stated that a single nonbinding futility analysis will be performed. With the option to perform 2 interim analyses for efficacy, the study team decided that a futility analysis may be performed at the time of each efficacy interim analysis.

7. REFERENCES

Klein, J.P., et al., Analyzing survival curves at a fixed point in time. Stat Med, 2007. 26(24): p. 4505-19.

8. APPENDICES

8.1. Power Estimates

Enrollment for the study will end November 1, 2021, regardless of the number of randomized participants accrued. Hence, the final number randomized will be known at the time of the first interim analysis. We computed power estimates for the following scenarios:

- Assume at the first interim that we will randomize 150, and final sample size = 150
- Assume at the first interim that we will randomize 150, and final sample size = 140

Table A1 presents power estimates for various combinations of RA-free survival rates for the placebo arm (45%, 50%, 55%) and the HCQ arm (70%, 75%, 80%). Based on prior information, the 50% vs 75% scenario represents a reasonable expectation. For the power estimates in Table A1, we simulated 10,000 trials under the following assumptions:

- Enrollment time of 5.5 years since study initiation
- 2 interim analyses conducted at 5.75 years and 6.75 years after study initiation
- Information proportions are calculated from the person-years estimates of study time since randomization, to a maximum of 3.0 years per participant, regardless of disease status or disposition, for each simulation sample
- LPLV at 8.5 years after study initiation
- Early Terminations/Lost to Follow-up participants: 20% in each treatment arm

The estimated overall power of the study equals the percent of simulated trials where the null hypothesis was rejected at an interim analysis or at the final end-of-study analysis.

Table A1: Overall Power Estimates for Various RA-Free Survival Rates

Final Sample Size	RA-Free Survival for Placebo	RA-Free Survival for HCQ		
		70%	75%	80%
150	45%	82.7%	94.4%	98.8%
	50%	64.8%	84.3%	95.7%
	55%	42.1%	66.9%	86.7%
140	45%	79.8%	92.9%	98.2%
	50%	60.6%	81.2%	94.3%
	55%	39.2%	63.2%	84.7%

Under the null hypothesis case (RA-free survival for both treatment arms at 50%), we estimated the following Type I error rates (95% CI), and they are statistically consistent with 5%:

- Final sample size = 150: 5.4% (5.0%, 5.8%)
- Final sample size = 140: 5.0% (4.6%, 5.4%)

Table A2 shows simulation results for the probability of meeting the futility criteria (Z-score<0.3).

Table A2: Probability of Finding Futility (Z-score<0.3)

Final Sample Size	Interim Analysis	RA-Free Survival for Placebo/HCQ	
		50%/75%	50%/50%
150	1st	1.2%	62.1%
	2nd	0.5%	62.1%
140	1st	1.5%	61.4%
	2nd	0.9%	60.9%

14.2. Schedule of Events

Table 6.1: Schedule of Events (Pre-Treatment through End of Treatment)

	Pre-Treatment	Eligibility		Subject Call (Week)	Evaluations Triggered by Swollen Joint	Treatment (Week)				End of Treatment (Week)	Time of Diagnosis Clinic Visit	Unscheduled Visit
Time Point	**Pre-screening**	**Screening**	**Baseline**	**18, 30 & 42**	**Any post-Baseline visit**	**6**	**12**	**24**	**36**	**52**		
Visit Windows (Days)		**-30 days**	**NA**	**±7 days**		**±7 days**				**±14 days**		
Clinical Blood Draw (mL)	**NA**	**22**	**NA**	**NA**	**NA**	**NA**	**NA**	**9**	**NA**	**9**	**NA**	**9**
Research Blood Draw (mL)	**5**	**5**	**66**	**NA**	**66**	**NA**	**NA**	**10**	**NA**	**66**	**66**	**NA**
Visit Draw Total (mL)	**5**	**27**	**66**	**NA**	**66**	**NA**	**NA**	**19**	**NA**	**75**	**66**	**9**
General Assessments												
Pre-Screening ICF	X[1]											
Pre-Screening Questionnaire	X											
Main Study ICF		X										
Demographics		X									X	
Medical History		X	X									
Prior/Concomitant Medications		X	X			X	X	X	X	X	X	X
Physical Exam[2]		X	X			X	X	X	X	X	X	X
Vital Signs including heart rate, sitting systolic/diastolic blood pressure, * include height (Screening only), weight, & waist circumference		X*	X			X	X	X	X	X	X*	X
NHYA Classification[3]		X										
Retinal Exam[4]		X[5]										
Randomization			X[6]									
AE/SAE Assessment						X	X	X	X	X	X	X
Profile 29 v2.0			X							X	X	
Epidemiologic Questionnaire			X							X[8]	X	
Dietary Assessment Questionnaire			X								X	
Self-Reported Joint Symptoms, including modified MDHAQ			X			X	X	X	X	X	X	X
Evaluate family member participation[7]			X			X	X	X	X	X	X	X
Pregnancy Status Check				X[8]		X[8]	X[8]	X[8]	X[8]	X[8]		X[8]
Treatment Telephone Assessments[9]				X[8]								
Annual Phone Call for Withdrawn Subjects										X[10]		
Disease Status												
Joint Examination – Physician's Assessment : 64 swollen joint count		X[11]										
Joint Examination – Physician's Assessment : 64 /66 tender/ swollen joint count[12]			X[11]			X[13]	X[13]	X[13]	X[13]	X[13]	X	X[13]
Anti-CCP3	X[14]	X										
Real Time Core Outcome Testing (hsCRP, IgM-RF, Anti-CCP3) & serum [15]			X		X[16]					X[17]		
Future Core Outcome Testing (hsCRP, IgM-RF, Anti-CCP3) & serum								X[8]			X	
2010 ACR/EULAR Criteria					X							
X-ray as needed[18]					X[18]							
Clinical Laboratory Assessments												
Screening Chemistries/Hematologies: Serum creatinine, ALT, AST, WBC, Platelets, ANC, Hemoglobin		X										
Infectious Disease Screen: HIV-1/HIV-2 Antigen/Antibody, Hep B Surface Antigen, Hep C Antibody		X[19]										
STAT Urine Pregnancy Test			X[20]		X[18]							
Chemistries: Serum creatinine, ALT, & AST								X[21]		X		X[21, 22]
Hematologies: Hemoglobin, Hematocrit, WBC (with differential), & Platelet count								X[21]		X		X[21, 22]
Required Mechanistic Specimens[16]												
PBMC/Plasma/DNA, RNA, Urine[23]			X		X[24]					X[8]	X	
HCQ Level[25] *(1 time draw during treatment period)*						X[26]	X[26]	X[26]	X[26]	X[26]	X[26]	X[26]
Study Product												
Dispense Study Product[27]			X[6]			X[8]	X[8]	X[8]	X[8]			
Pill Count						X[8]	X[8]	X[8]	X[8]	X[8]	X	

[1] Subjects identified through the first degree relative or general population recruitment strategies **will** be given, and subjects identified through a review of clinic records **may** be given, a Pre-Screening consent form to indicate their consent to undergo Pre-Screening procedures as described in Section 6.5.1, *Pre-Screening*.

[2] Physical Exam: Full PE at Screen, Week 52, Time of Diagnosis and Month 36/Early Termination. Symptom-driven PE at all other clinic visits.

[3] NHYA Classification: See Section 15.4, *NYHA Classification*.

[4] If a subject develops ocular symptoms, the subject will be referred to clinical care (see section 5.8.1, *Study Treatment Discontinuation)*.

[5] A retinal exam by an ophthalmologist or optometrist that includes a dilated funduscopic exam, visual field (10-2) and OCT should be conducted prior to the Baseline visit (after all other screening eligibility criteria have been confirmed). *Note: Results from the initial screening visit retinal examination may be used to assess eligibility for up to 6 months.*

[6] **Subjects with swollen joints that are consistent with RA-like synovitis at the time of the Baseline/Randomization Visit should NOT be randomized or treated. Sites utilizing a central pharmacy may randomize subjects prior to the Baseline visit after initial eligibility at Screening has been confirmed, but if swollen joints that are consistent with RA-like synovitis are noted at baseline, do NOT dispense study therapy to the subject**. *Note: The first dose of study therapy will be given in clinic. The subject will be observed per institutional standards.*

[7] All participants should complete the "Participation of First Degree Relatives" questionnaire. If the subject consents to linking his/her information with a participating family members, please follow the Process for Linking First Degree Relatives in the Manual of Operations.

[8] These procedures will be conducted if the subject **has not** been previously diagnosed with clinically-apparent RA.

[9] Telephone Assessment: Coordinators will call subjects at Week 18, 30, and 42 to assess toxicities to HCQ, AEs, a review of study drug dosing and storage, joint symptoms, pregnancy status, answer subject questions, and confirm contact information.

[10] Subjects who have withdrawn from the study and consent to annual phone calls will be asked about development of RA and related information.

[11] **If a subject has a swollen joint consistent with RA-like synovitis, do not randomize or dispense study drug to the subject.**

[12] Note: The count will include 66 tender/64 swollen joints. The midfoot joints will not be evaluated for either tenderness or swelling. The hip joints will not be evaluated for swelling.

[13] If the subject has 1 or more swollen joints that are consistent with RA-like synovitis, then complete additional assessments as outlined in section 6.5.9, *Evaluations Triggered by a Swollen Joint*.

[14] If a subject found through the rheumatology clinic has already had Anti-CCP levels assessed in the previous 12 months, historical results may be used to assess eligibility at Pre-Screening. Pre-Screening specimens will be analyzed at the site local laboratory.

[15] Results from real time core outcome testing specimens will be reported back to the sites for review of the 2010 ACR/EULAR criteria.

[16] Collection of core outcome test and mechanistic studies specimens will be discontinued after a subject has been diagnosed with clinically-apparent RA and the Time of Diagnosis visit has been completed. Subjects who have swollen joints consistent with RA-like synovitis with no erosions will follow the normal schedule for core outcome testing collection.

[17] Subjects who have been previously diagnosed with clinically-apparent RA will have hsCRP (only) assessed at Week 52.

[18] X-rays may be conducted every 6 months if needed. All subjects with swollen joints consistent with RA-like synovitis will have at least 1 x-ray but follow-up x-rays are not needed if clinically-apparent RA is diagnosed (see Section 3.1, *Description of Study Design*). At most, a subject may undergo study-related x-ray imaging 4 times throughout his/her participation in the study. Female subjects who have child bearing potential cannot undergo x-ray imaging unless a STAT urine pregnancy test is negative.

[19] If any of the infectious disease tests yield a positive result, consult exclusion criteria in Section 4.2, *Exclusion Criteria*, for subject eligibility. The site will report these results to the subject and perform other follow-up per institutional guidelines.

[20] Female subjects cannot receive the initial dose of study product until eligibility can be confirmed via a STAT urine pregnancy test.

[21] Note: Abnormal lab values meeting the criteria noted in Section 5.8.1, *Study Treatment Discontinuation*, should be re-tested within 4 weeks, prior to discontinuation.

[22] If needed for evaluation of safety related to drug toxicity during the treatment period, the chemistry and hematology draws will be collected.

[23] DNA collection is optional. This specimen will be collected at the Baseline visit for subjects who consent to the collection for analysis of the shared epitope. DNA will be collected at subsequent visits for subjects who consent to future genetic testing.

[24] Specimens for mechanistic studies will be collected at the **initial** finding of RA-like synovitis.

[25] It is strongly recommended that the HCQ level specimen be drawn 4 or more hours after the last HCQ dose.

[26] The HCQ level specimen will be collected at one time point for each subject. The specimen collection may occur at the Week 24, Week 52, or at any visit where study therapy is discontinued (including Time of Diagnosis and Early Termination visits) provided the specimen was not collected previously.

[27] Please refer to protocol section 5.3.2, *Administration*, for the study therapy distribution schedule.

Table 6.2: Schedule of Events (Follow-Up)

	Subject Call (Months)	Evaluations Triggered by a Swollen Joint[28]	Follow-Up (Months)			End of Study (Month)	Time of Diagnosis Clinic Visit	Unscheduled Visit
Time Point	**15, 21, 27, & 33**	**At any visit**	**18**	**24**	**30**	**36/ Early Termination**		
Visit Windows (Days)	**±14 days**		**±14 days**					
Clinical Blood Draw (mL)	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
Research Blood Draw (mL)	**NA**	**66**	**10**	**10**	**10**	**66**	**66**	**NA**
Visit Draw Total (mL)	**NA**	**66**	**10**	**10**	**10**	**66**	**66**	**NA**
General Assessments								
Demographics						X	X	
Concomitant Medications			X	X	X	X	X	X
Physical Exam[29]			X	X	X	X	X	X
Vital Signs including heart rate & sitting systolic/diastolic blood pressure				X				X
Vital Signs including heart rate, sitting systolic/diastolic blood pressure, weight, & waist circumference						X	X	
AE/SAE Assessment			X[30]	X[30]	X[30]	X[30]		X[30]
Profile 29 v2.0				X		X	X	
Epidemiologic Questionnaire				X[31]		X[31]	X	
Dietary Assessment Questionnaire						X[31]	X	
Self-Reported Joint Symptoms, including modified MDHAQ			X	X	X	X	X	X
Evaluate family member participation[32]			X	X	X	X	X	X
Pregnancy Status Check			X[31]	X[31]	X[31]	X[31]		X[31]
Follow-up Telephone Assessments[33]	X[31]							
Annual Phone Call for Withdrawn Subjects				X[34]		X[34]		
Disease Status								
Joint Examination – Physician's Assessment: 64 /66 tender/swollen joint count[35]			X[36]	X[36]	X[36]	X[36]	X	X[36]
Real Time Core Outcome Testing (hsCRP, IgM-RF, Anti-CCP3) & serum[37]		X[38]				X[39]		
Future Core Outcome Testing (hsCRP, IgM-RF, Anti-CCP3) & serum			X[31]	X[31]	X[31]		X	
2010 ACR/EULAR Criteria [40]		X						
X-ray as needed [41]		X[41]						
Clinical Laboratory Assessments								
Hematologies: Hemoglobin, Hematocrit, WBC (with differential), & Platelet count								X[42]
Chemistries: Serum creatinine, ALT, & AST								X[42]
STAT Urine Pregnancy Test		X[41]						
Required Mechanistic Specimens[31]								
PBMC/Plasma/DNA, RNA, Urine[43]		X[44]				X[31]	X	
HCQ Level[45]						X[46]	X[46]	

[28] See protocol section 6.5.9, *Evaluations Triggered by a Swollen Joint*, for additional details.

[29] Physical Exam: Full PE at Screen, Week 52, Time of Diagnosis and Month 36/Early Termination. Symptom-driven PE at all other clinic visits.

[30] Non-serious adverse events will not be collected after Month 18. SAEs will be collected for the duration of the study.

[31] These procedures will be conducted if the subject **has not** been previously diagnosed with clinically-apparent RA.

[32] All participants should complete the "Participation of First Degree Relatives" questionnaire. If the subject consents to linking his/her information with a participating family members, please follow the Process for Linking First Degree Relatives in the Manual of Operations.

[33] Follow-up Telephone Assessments: Coordinators will call subjects at Months 15, 21, 27, and 33 to assess AEs (until Month 15); joint symptoms, answer subject questions, and confirm contact information.

[34] Subjects who have withdrawn from the study and consent to annual phone calls will be asked about development of RA and related information.

[35] Note: The count will include 66 tender/64 swollen joints. The midfoot joints will not be evaluated for either tenderness or swelling. The hip joints will not be evaluated for swelling.

[36] If the subject has 1 or more swollen joints that are consistent with RA-like synovitis, then complete additional assessments as outlined in section 6.5.9, *Evaluations Triggered by a Swollen Joint*.

[37] Results from real time core outcome testing specimens will be reported back to the sites for review of the 2010 ACR/EULAR criteria.

[38] Collection of core outcome tests and mechanistic studies specimens will be discontinued after a subject has been diagnosed with clinically-apparent RA and the Time of Diagnosis visit has been completed. Subjects who have swollen joints consistent with RA-like synovitis with no erosions will follow the normal schedule for core outcome testing collection.

[39] Subjects who have been previously diagnosed with clinically-apparent RA will only have hsCRP assessed at this time point.

[40] Review the 2010 ACR/EULAR Criteria results if subject has 1 or more swollen joints that are consistent with RA-like synovitis.

[41] X-rays may be conducted every 6 months if needed. All subjects with swollen joints consistent with RA-like synovitis will have at least 1 x-ray but follow-up x-rays are not needed if clinically-apparent RA is diagnosed (see Section 3.1, *Description of Study Design*). At most, a subject may undergo study-related x-ray imaging 4 times throughout his/her participation in the study. Female subjects who have child bearing potential cannot undergo x-ray imaging unless a STAT urine pregnancy test is negative.

[42] Chemistry and hematology assessments may be performed, if needed.

[43] DNA will be collected for subjects who consent to future genetic testing.

[44] Specimens for mechanistic studies will be collected at the **initial** finding of RA-like synovitis.

[45] It is strongly recommended that the HCQ level specimen be drawn 4 or more hours after the last HCQ dose.

[46] The HCQ level specimen will be collected at one time point for each subject. The specimen collection may occur at the Week 24, Week 52, or at any visit where study therapy is discontinued (including Time of Diagnosis and Early Termination visits)provided the specimen was not collected previously.

ACKNOWLEDGEMENT AND SIGNATURE SHEET

Approved: DocuSigned by:
Signer Name: Kevin Deane
Signing Reason: I approve this document
Signing Time: 12-May-2023 | 12:25:31 PM EDT
0CBDBD077DAF4126AC2597245B3BD928
Date: _____

Kevin Deane, MD, PhD
Protocol Chair

Approved: DocuSigned by: [redacted] Date: _____

[redacted]
Medical Monitor

Approved: [redacted] Date: _____

[redacted]
Scientist

Approved: DocuSigned by: [redacted] Date: _____

[redacted]
Statistician

DocuSign

Certificate Of Completion

Envelope Id: 3C7300B3C3894D7C993B66D30D6805D3
Status: Completed
Subject: Complete with DocuSign: ARA08 Statistical Analysis Plan v1.0 2023-05-08
Source Envelope:
Document Pages: 1
Signatures: 4
Envelope Originator:
Certificate Pages: 5
Initials: 0
[redacted]
AutoNav: Enabled
EnvelopeId Stamping: Disabled
Time Zone: (UTC-05:00) Eastern Time (US & Canada)

Record Tracking

Status: Original
5/8/2023 1:42:32 PM
Holder: [redacted]
Location: DocuSign
Security Appliance Status: Connected
Pool: FedRamp
Storage Appliance Status: Connected
[redacted]
Location: DocuSign

Signer Events	Signature	Timestamp
[redacted] Security Level: Email, Account Authentication (Required)	[redacted] Signature Adoption: Pre-selected Style Signature ID: F5164821-34FC-4FDD-AF32-88BD039F6ECC Using IP Address: 156.40.216.7 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 5/8/2023 1:47:53 PM Viewed: 5/9/2023 9:36:47 AM Signed: 5/9/2023 9:37:11 AM
Electronic Record and Signature Disclosure: Accepted: 8/22/2020 3:45:44 PM ID: 5626e8be-940c-4427-b70c-5a067a412344		
Kevin Deane KEVIN.DEANE@CUANSCHUTZ.EDU Security Level: Email, Account Authentication (Required)	 Signature Adoption: Drawn on Device Signature ID: 0CBDBD07-7DAF-4126-AC25-97245B3BD928 Using IP Address: 140.226.25.93 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 5/8/2023 1:47:53 PM Viewed: 5/12/2023 11:05:59 AM Signed: 5/12/2023 12:25:39 PM
Electronic Record and Signature Disclosure: Accepted: 12/21/2021 4:44:18 PM ID: d5c7524b-2f11-4a5b-8e7f-7566a0b3df1d		

Signer Events	Signature	Timestamp
[redacted] Security Level: Email, Account Authentication (Required)	[redacted] Signature Adoption: Pre-selected Style Signature ID: 2092CB4E-55B8-43D7-8F00-CD7B1C839C76 Using IP Address: 4.34.23.236 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 5/8/2023 1:47:53 PM Viewed: 5/10/2023 2:23:19 PM Signed: 5/10/2023 2:24:16 PM
Electronic Record and Signature Disclosure: Accepted: 5/10/2023 2:23:19 PM ID: 9b2fd60e-be32-4121-9e88-428861ae4ed5		
[redacted] Security Level: Email, Account Authentication (Required)	[redacted] Signature Adoption: Pre-selected Style Signature ID: EF28359E-69A2-4300-934F-0C21D39AF564 Using IP Address: 4.34.23.236 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I am the author of this document	Sent: 5/8/2023 1:47:53 PM Viewed: 5/8/2023 1:48:07 PM Signed: 5/8/2023 1:48:30 PM
Electronic Record and Signature Disclosure: Not Offered via DocuSign		

In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	5/8/2023 1:47:54 PM
Certified Delivered	Security Checked	5/8/2023 1:48:07 PM
Signing Complete	Security Checked	5/8/2023 1:48:30 PM
Completed	Security Checked	5/12/2023 12:25:39 PM

Payment Events	Status	Timestamps

Electronic Record and Signature Disclosure

ELECTRONIC RECORD AND SIGNATURE DISCLOSURE

From time to time, [redacted]. (we, us or Company) may be required by law to provide to you certain written notices or disclosures. Described below are the terms and conditions for providing to you such notices and disclosures electronically through the DocuSign system. Please read the information below carefully and thoroughly, and if you can access this information electronically to your satisfaction and agree to this Electronic Record and Signature Disclosure (ERSD), please confirm your agreement by selecting the check-box next to 'I agree to use electronic records and signatures' before clicking 'CONTINUE' within the DocuSign system.

Getting paper copies

At any time, you may request from us a paper copy of any record provided or made available electronically to you by us. You will have the ability to download and print documents we send to you through the DocuSign system during and immediately after the signing session and, if you elect to create a DocuSign account, you may access the documents for a limited period of time (usually 30 days) after such documents are first sent to you. After such time, if you wish for us to send you paper copies of any such documents from our office to you, you will be charged a $0.00 per-page fee. You may request delivery of such paper copies from us by following the procedure described below.

Withdrawing your consent

If you decide to receive notices and disclosures from us electronically, you may at any time change your mind and tell us that thereafter you want to receive required notices and disclosures only in paper format. How you must inform us of your decision to receive future notices and disclosure in paper format and withdraw your consent to receive notices and disclosures electronically is described below.

Consequences of changing your mind

If you elect to receive required notices and disclosures only in paper format, it will slow the speed at which we can complete certain steps in transactions with you and delivering services to you because we will need first to send the required notices or disclosures to you in paper format, and then wait until we receive back from you your acknowledgment of your receipt of such paper notices or disclosures. Further, you will no longer be able to use the DocuSign system to receive required notices and consents electronically from us or to sign electronically documents from us.

All notices and disclosures will be sent to you electronically

Unless you tell us otherwise in accordance with the procedures described herein, we will provide electronically to you through the DocuSign system all required notices, disclosures, authorizations, acknowledgements, and other documents that are required to be provided or made available to you during the course of our relationship with you. To reduce the chance of you inadvertently not receiving any notice or disclosure, we prefer to provide all of the required notices and disclosures to you by the same method and to the same address that you have given us. Thus, you can receive all the disclosures and notices electronically or in paper format through the paper mail delivery system. If you do not agree with this process, please let us know as described below. Please also see the paragraph immediately above that describes the consequences of your electing not to receive delivery of the notices and disclosures electronically from us.

How to contact [redacted]

You may contact us to let us know of your changes as to how we may contact you electronically, to request paper copies of certain information from us, and to withdraw your prior consent to receive notices and disclosures electronically as follows:
To contact us by email send messages to: [redacted]

To advise [redacted] of your new email address

To let us know of a change in your email address where we should send notices and disclosures electronically to you, you must send an email message to us at [redacted] and in the body of such request you must state: your previous email address, your new email address. We do not require any other information from you to change your email address.

If you created a DocuSign account, you may update it with your new email address through your account preferences.

To request paper copies from [redacted]

To request delivery from us of paper copies of the notices and disclosures previously provided by us to you electronically, you must send us an email to [redacted] and in the body of such request you must state your email address, full name, mailing address, and telephone number. We will bill you for any fees at that time, if any.

To withdraw your consent with [redacted]

To inform us that you no longer wish to receive future notices and disclosures in electronic format you may:

i. decline to sign a document from within your signing session, and on the subsequent page, select the check-box indicating you wish to withdraw your consent, or you may;

ii. send us an email to [redacted] and in the body of such request you must state your email, full name, mailing address, and telephone number. We do not need any other information from you to withdraw consent.. The consequences of your withdrawing consent for online documents will be that transactions may take a longer time to process..

Required hardware and software

The minimum system requirements for using the DocuSign system may change over time. The current system requirements are found here: https://support.docusign.com/guides/signer-guide-signing-system-requirements.

Acknowledging your access and consent to receive and sign documents electronically

To confirm to us that you can access this information electronically, which will be similar to other electronic notices and disclosures that we will provide to you, please confirm that you have read this ERSD, and (i) that you are able to print on paper or electronically save this ERSD for your future reference and access; or (ii) that you are able to email this ERSD to an email address where you will be able to print on paper or save it for your future reference and access. Further, if you consent to receiving notices and disclosures exclusively in electronic format as described herein, then select the check-box next to 'I agree to use electronic records and signatures' before clicking 'CONTINUE' within the DocuSign system.

By selecting the check-box next to 'I agree to use electronic records and signatures', you confirm that:

- You can access and read this Electronic Record and Signature Disclosure; and
- You can print on paper this Electronic Record and Signature Disclosure, or save or send this Electronic Record and Disclosure to a location where you can print it, for future reference and access; and
- Until or unless you notify [redacted] as described above, you consent to receive exclusively through electronic means all notices, disclosures, authorizations, acknowledgements, and other documents that are required to be provided or made available to you [redacted] during the course of your relationship with [redacted]