Treatments Against RA and Effect on FDG PET-CT: The TARGET Trial

Clinical Trial Protocol

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STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6) and the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). All personnel involved in the conduct of this study have completed human subjects protection training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator or Co-Principal Site Investigators:

Signed: _____ Date: _____

Name:

Title:

Signed: _____ Date: _____

Name:

Title:

LIST OF ABBREVIATIONS

BWH	Brigham and Women's Hospital
CRF	Case Report Form
CUMC	Columbia University Medical Center
DAS	Disease Activity Score
DCC	Data Coordinating Center
DSMB	Data and Safety Monitoring Board
EDC	Electronic Data Capture
eCRF	Electronic Case Report Form
EULAR	European League Against Rheumatism
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
IRB	Institutional Review Board
LDAR	Low Disease Activity or Remission
MHDA	Moderate or High Disease Activity
MOOP	Manual of Operating Procedures
N	Number (typically refers to subjects)
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PHI	Protected Health Information
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
TARGET	Treatments Against RA and Effect on FDG PET/CT Trial
UP	Unanticipated Problem

PROTOCOL SUMMARY

Title:

Treatments Against RA and Effect on FDG PET-CT:

The TARGET Trial

Précis:

The *overarching goal* of this trial is to examine the effect of RA disease modifying drugs (DMARDs) on vascular inflammation. We will accomplish this goal by comparing two treatment regimens for RA in the setting of an RCT in patients with inadequate response to methotrexate (MTX). These are: a tumor necrosis factor inhibitor (TNFi) + MTX + hydroxychloroquine (HCQ [if subject was taking HCQ at study entry]) versus triple therapy (MTX + sulfasalazine [SSZ] + hydroxychloroquine [HCQ]). The trial design is a two-arm RCT with blinded joint assessment and blinded FDG PET/CT readers. Subjects and rheumatologists will be unblinded to treatment assignment but will be blinded to FDG PET/CT results. Joint count assessors will be blinded to treatment assignment. The trial will be conducted at multiple sites across the US, with a duration of six months. Recruitment will be over 24 months with six more months of follow-up.

Objectives:

Primary: **To compare the effects on vascular inflammation of TNFi + MTX versus triple therapy in subjects with RA who are inadequate responders to MTX using FDG PET/CT as a tool for detecting vascular (arterial) inflammation.**

Secondary: **To compare the effects on vascular inflammation of achieving low disease activity or remission (LDAR) vs moderate-high disease activity (MHDA).**

Exploratory Aim 1: To determine researchers' attitudes and beliefs regarding their ethical obligation to return and manage incidental research findings from whole body FDG PET/CT imaging, including what to report, when to report, and how to report, as well as how to manage and to what extent.

Exploratory Aim 2: To determine the prevalence of incidental research findings on whole body FDG PET/CT in RA and detection rate of previously unknown malignancies.

Exploratory Aim 3: To investigate associations between stress-associated neurobiological activity and articular and arterial treatment response in RA.

Population:	Subjects for this trial will be RA patients who are deemed methotrexate-inadequate responders (MTX-IRs, DAS28>3.2) by their treating rheumatologist, and who have not yet started treatment with a biologic DMARD and are currently not receiving any other DMARD than MTX or MTX and HCQ. In order to enrich our trial subjects for atherosclerosis, male RA patients must be at least 45 years old, and women at least 50 years old. Additional inclusion and exclusion criteria are listed in Section 3 of this document.
Phase:	Phase IV
Number of Sites:	This will be a multicenter study. The lead sites will be Brigham & Women's Hospital and Columbia University Medical Center. Participating sites will be selected based on the following criterion: adequate number of RA patients**;** access to an FDG-PET scanner; availability of trial staff (i.e. coordinator, nurse, trained joint count assessor (metrologist)**;** and experience enrolling and following subjects in previous clinical trials**.**
Description of Intervention:	Enrolled subjects will be asked to take 6 months of RA medication according to the treatment arm to which they are randomized, which will be either be triple therapy or a TNFi. Subjects will be evaluated every six weeks for clinical response. For subjects receiving a TNFi, if good treatment response (CDAI ≤10) is not achieved by 18 weeks, then treatment will be adjusted. For subjects receiving adalimumab, this medication will be discontinued and switched to etanercept 50 mg every week. If the subject is receiving etanercept, this medication will be discontinued and switched to adalimumab 40 mg every other week. Subjects will remain on this new medication until the end of the study. Subjects assigned to the <u>triple therapy arm</u> will begin SSZ 500 mg twice daily and HCQ 200 mg twice daily, not to exceed 6.5 mg/kg. At 6 weeks, the dose of SSZ will increase from 500 mg twice daily to 1 g twice daily for all patients. At 18 weeks, if a <u>good treatment response</u> (CDAI ≤ 10) has not been achieved, MTX will be stopped and leflunomide 20 mg daily started. The participant will remain on leflunomide (and concomitant SSZ + HCQ) for the remaining six weeks of the study. As part of a bioethics ancillary study, we will survey the TARGET investigators to determine researchers' attitudes and beliefs regarding

their ethical obligation to return and manage incidental research findings from whole body PET/CT imaging studies and determine the prevalence of incidental extra cardiac findings on whole body PET/CT scans in rheumatoid arthritis (RA) and detection rate of previously unknown malignancies.

Study Duration: 48 months

Subject Participation Duration: 6 months

Estimated Time to Complete Enrollment: 24 months

FIGURE 1. STUDY DESIGN

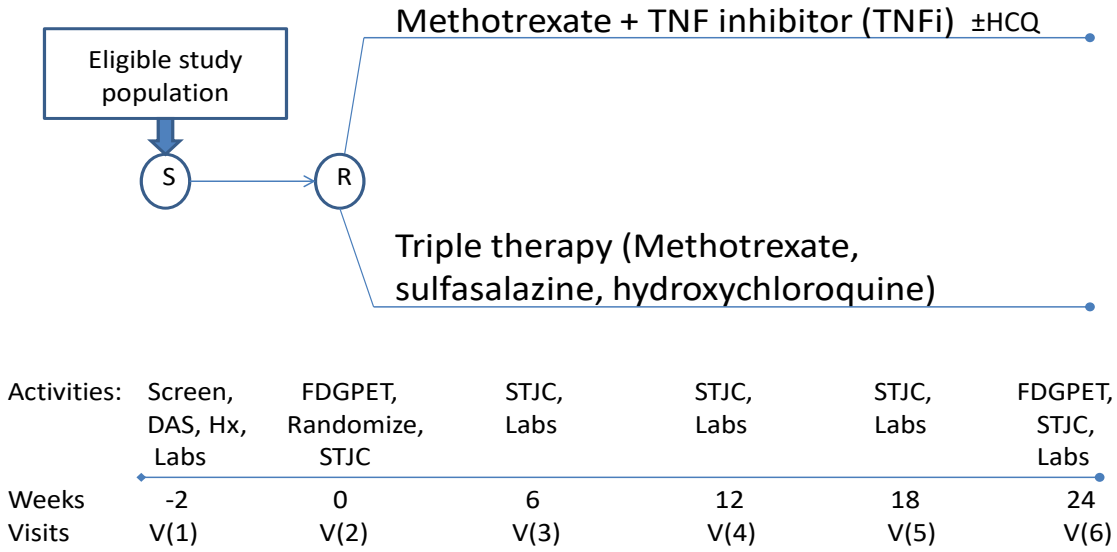
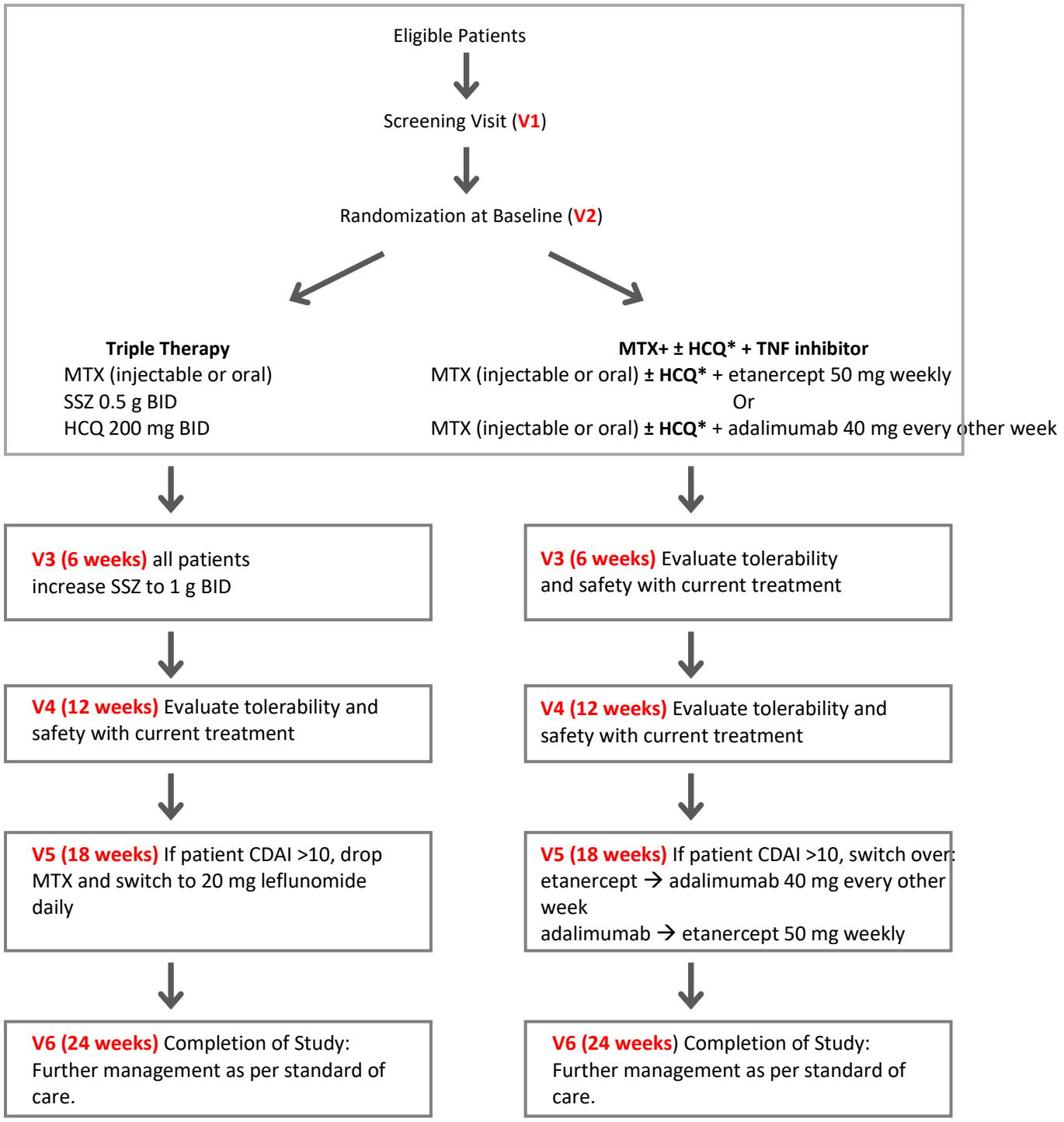


TABLE 1. SCHEDULE OF STUDY VISITS AND PROCEDURES

Table 1: Visit Schedule	Screen V_1 [Wk-2]	Scan 1 [Wk-1]	Call to Randomize [Wk-0.5]	Baseline V_2 [Wk0]	V_3 [Wk6]	V_4 [Wk12]	V_5 [Wk18]	V_6 [Wk24]
Eligibility & consent	x							
CRP	x							
TB, HBV, HCV test	x							
CXR	x							
HbA1c (Type II Diabetics only)	x							
FDG PET/CT		x						x
Pregnancy test		x						x
Glucose test		x						x
Randomize			x					
Blood pressure/Clinical Parameters	x				x	x	x	x
CBC, CMP (or LFTs and BMP)	x				x	x	x	x
Biospecimen collection				x	x		x	x
Joint count	x			x	x	x	x	x
Questionnaires	x			x	x	x	x	x

Visits shall occur ± 2 weeks according to the visit schedule

FIGURE 2. TREATMENT ALGORITHM



* Subjects entering the study on concomitant HCQ and assigned to the TNFi arm will continue to take HCQ at its original dose.

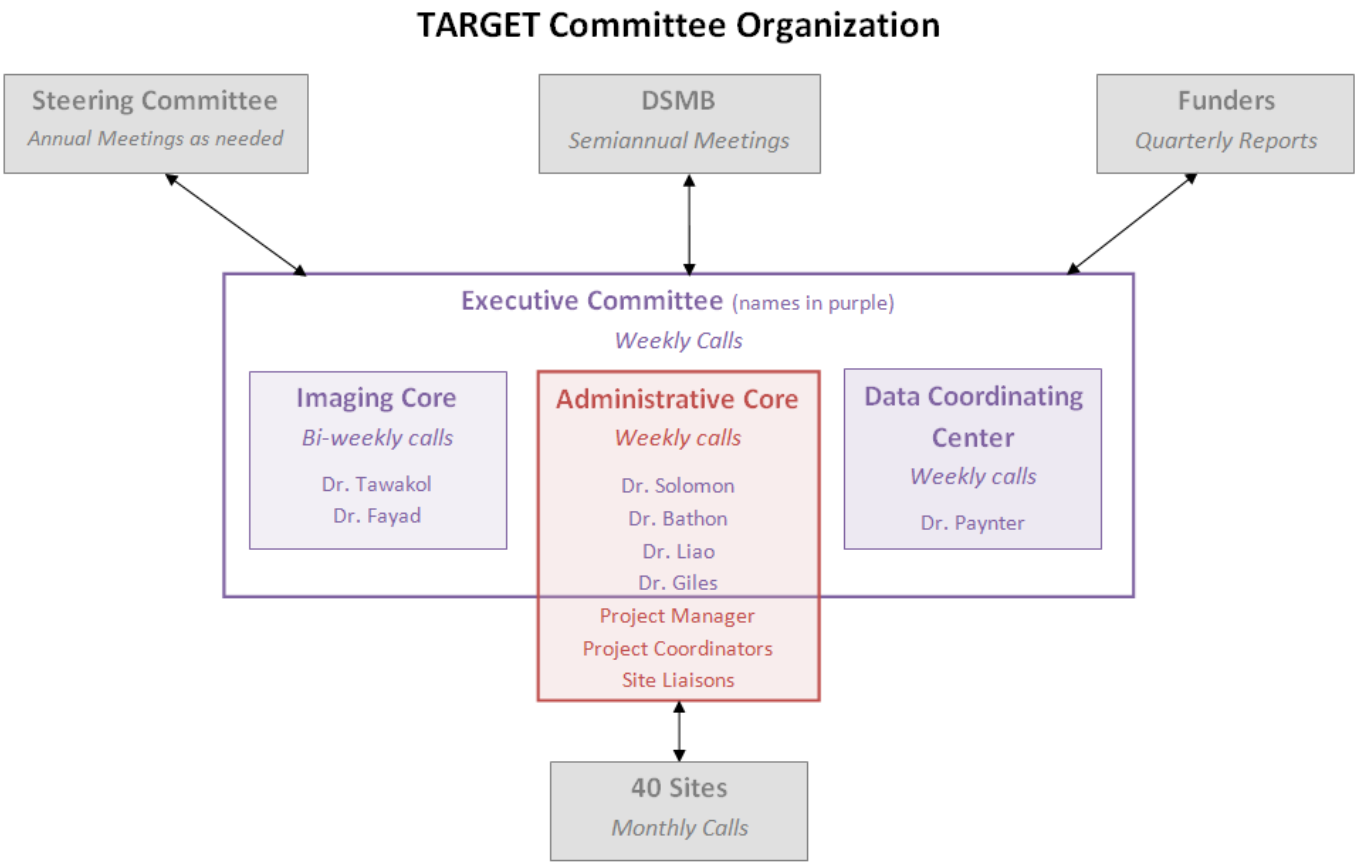
**Subjects entering the study on concomitant HCQ at <200mg BID who are assigned to triple therapy will increase their HCQ dose to 200mg BID, provided this new dose does not exceed 6.5mg/kg.

1. KEY ROLES AND CONTACT INFORMATION

1.1 Study Organization and Roster

A diagram of the study organization is shown below. Each of the individual elements is discussed in the following sections.

FIGURE 3: STUDY ORGANIZATION



1.1.1 Study Staff Roster

For a list of the TARGET PIs, co-investigators, and key study staff see the MOOP.

1.2 Administrative Core

The Administrative Core consists of the TARGET PIs (Drs. Solomon and Bathon), as well as their co-investigators (Drs. Giles and Liao), Dr. Lu (Project Manager), Mr. Zartoshti (Project Coordinator), Ms. Donnelly (Grants Administrator, BWH), and research assistants and coordinators whole will act as TARGET site liaisons. It will be run jointly by Dr. Solomon from BWH and Dr. Bathon of CUMC. This Core is responsible for overseeing study administration. The responsibilities of the Administrative Core include:

- Development and maintenance of the Clinical Trial Protocol and MOOP
- Assuring the study is conducted according to the Protocol and MOOP
- Communications with clinical sites, scheduling of meetings and training sessions, responding to and documenting ad hoc communications
- Distribution of all changes, updates and policies of reports and documents to participating clinical sites
- Maintaining the study binder (regulatory and clinical documents)
- Participating in protocol finalization and preparing study materials

1.3 Executive Committee

This committee will be made up of the co-PIs; Dr. Giles and Dr. Liao; as well as Dr. Rist, PhD who leads the DCC; and Dr. Tawakol and Dr. Fayad, of the Imaging Core. This committee will meet weekly for the first six months of the trial, then monthly thereafter. Any concerns voiced by other study staff members will be brought to the attention of this committee. The progress of the study will be reviewed by the Executive Committee, and any issues will be identified and resolved through this Committee. The executive committee will be responsible for making strategic decisions regarding study protocol, resource allocation, recruitment, and protocol adherence. They will have final say on decisions for the entire trial. If conflicts arise between the Co-PIs, issues will be brought to the entire Executive Committee for resolution.

1.4 Steering Committee

The Steering Committee will meet regularly to discuss progress of the trial and provide critical feedback to the Executive Committee regarding clinical, conceptual, methodological, and feasibility issues that arise through the duration of the trial. The Steering Committee will include the two Co-PIs (Joan Bathon and Dan Solomon) and several key co-Investigators.

1.5 Imaging Core

The Imaging Core will be co-led by Dr. Tawakol (Massachusetts General Hospital) and Dr. Fayad (Mt. Sinai School of Medicine). They will be responsible for overseeing all matters related to collection and assessment of the FDG-PET/CT readings. This will include training imaging sites in standardized protocol techniques, monitoring consistency and quality of the scans, managing transmission and assessment of scans, and facilitating corrective actions for imaging sites as needed. Drs. Tawakol and Fayad will sit on the Executive Committee to ensure communication between the radiologists and cardiologists and to ensure seamless resolution of any technical and quality assurance issues.

1.6 Data Coordinating Center

The Data Coordinating Center (DCC) will be run by Pamela Rist, PhD of the Division of Preventive Medicine at Brigham and Women's Hospital. Robert Glynn, PhD, ScD will serve as Senior Biostatistician. The DCC is responsible for oversight of data collection and analysis. The responsibilities of the DCC include:

- Randomization scheme and procedures
- Development and implementation of the data flow, schedules for transferring data from sites, and data tracking
- Development of procedures for data entry, error identification, and error correction

- Adverse event monitoring and reporting to the DSMB
- Site monitoring via the Electronic Data Capture (EDC) to ensure adherence to the protocol and procedures
- Quality control procedures
- Creating reports - enrollment, adverse events, participant status (e.g., withdrawals) by site
- Trial analyses

1.7 Data and Safety Monitoring Board

The DSMB is discussed in more detail in Section 8.2 of this document. Briefly, the DSMB will be appointed by the NIH. We anticipate that its membership will include: a trial biostatistician; a cardiologist with trial experience; several rheumatologists with trial experience; and a bioethicist. The DSMB will need to approve the study protocol prior to commencement. The DSMB will meet every 6-12 months during the trial to review the conduct of the trial and the safety experience of subjects. Blinded (and if necessary unblinded) reports will be provided to them by the DCC.

1.8 Funding Sources

The NIH will provide the majority of funds for this trial. These funds will be supplemented by funds raised by the fNIH. As stakeholders of this trial, we anticipate that funders will have a responsibility in the oversight of the trial; specific arrangement may differ by funding agency. They will be updated at regular timepoints with reports of trial progress such as recruitment status, unforeseen problems, and updates to specimen collection.

1.9 Central IRB

The Partners HealthCare IRB will play the role of central IRB for this trial. Those sites that are able to rely on the central IRB will do so through the signing of a reliance agreement. Those sites unable to use a central IRB will be required to obtain their institutional approval for conduct of the trial. The Administrative Core and Executive Committee will be responsible for handling all communication with the central IRB. The DCC will provide all necessary reports for submission to the central IRB.

1.10 Clinical Sites

Approximately forty clinical sites will participate in enrolling subjects for the TARGET study. The study sites play an essential role in answering the questions posed by this study. Each site will be responsible for enrolling participants, carrying out the study protocol, recording and transmitting study information, satisfying regulatory requirements, and providing clinical oversight. The DCC and Administrative Core will support Sites in these efforts, from training through implementation. As well, the Imaging Core will support the FDG PET/CT acquisition.

Site Requirements for Eligibility include:

- Prior experience with clinical trials
- At least 150 active RA patients
- Easy access to FDG-PET CT imaging acquisition facility
- Easy access to standard clinical laboratory assessments
- Joint count assessor

The Roles and Responsibilities of the PI, co-PIs and Study Coordinator are outlined in the MOOP, Section 2.

1.11 Imaging Sites

All of the clinical sites are in close proximity to an imaging facility with a PET/CT scanner. The TARGET Imaging Core will evaluate each facility first to ensure that it has the necessary equipment and data transfer capabilities, and will then embark on a Site Initiation procedure. Once the local imaging site qualifies, training on the TARGET protocol will ensue.

Sites will transmit the completed scan data via the FTP System at the Imaging Core. Quality control/assurance procedures will be managed by the Imaging Core.

1.12 Laboratories

1) Safety laboratory studies drawn at Visits 1 and 3-6 will be assayed at local labs.

2) The Biorepository at the BWH Division of Preventive Medicine will be responsible for overseeing procurement and processing of all sera and plasma biospecimens. Whole and frozen blood will be sent directly from clinical sites to the biorepository. Sera and plasma will be spun and frozen in 1 ml aliquots and stored at -80C at the biorepository. The aliquots stored in the Biorepository are being collected in expectation of considerable interest in Ancillary Studies. The Biorepository will be responsible for retrieval of aliquots needed for the proteomic assays, the assays performed at the Harvard CTSA, and for funded ancillary proposals involving biomarkers.

3) The Harvard CTSA laboratory will perform assays of analytes collected at visits 2, 3, 5, and 6 that will be used to assess several CV risk factors (lipids, insulin, and glucose). These assays will take place at the end of the study on batched samples. This will assure uniformity in these measurements.

4) Crescendo/Myriad has agreed to perform two proteomic assays (VECTRA DA and RBM Discovery Panel 3.3) on aliquots from visit 2, visit 3, visit 5, and visit 6.

2. BACKGROUND AND RATIONALE

2.1 Background Information

2.1.1 Patients with RA are at increased risk for CVD.

RA is one of the most common autoimmune rheumatic conditions, affecting 0.5-1.0% of the adult population[1,2]. Remarkable progress in the treatment of RA has occurred in the past 2 decades with earlier and more aggressive treatment utilizing combinations of non-biologic and newer targeted biologic agents[3-5], resulting in less disability and fewer joint replacements in RA[6]. RA also has significant adverse effects on other organ systems, most notably the CV system. RA is associated with a 1.5-3-fold increase in mortality compared to non-RA controls[7], with CVD consistently the leading cause of these excess deaths[8]. The direct cost of CVD in RA in the US is estimated at $500M per year[9]. In contrast to the successful management of articular disease in RA, mortality rates have not declined despite marked improvements in survival in the general US population over the past 2-3 decades[10]. Rates of non-fatal CV events such as myocardial infarction (MI) and stroke, as well as the prevalence of subclinical atherosclerosis in coronary and carotid arterial beds are also higher in RA compared to non-RA controls[11,12]. Management of CVD risk in RA is based on general population guidelines without tailoring. *These data emphasize the urgency of developing strategies to identify and better manage the increased CV risk in RA.*

2.1.2 The increased CV risk in RA is likely due to enhanced vascular and/or systemic inflammation.

Atherosclerosis in the general population is recognized as a low-grade inflammatory process[13], as reflected by the presence of macrophages, T and B cells in plaque, and the association of plaque with elevated circulating levels of inflammatory cytokines[14]. 'Vulnerable plaque' likely to rupture is characterized by increased numbers of activated inflammatory cells, particularly macrophages. These macrophages elaborate matrix metalloproteinases (MMPs) that in turn erode the plaque's fibrous cap leading to extrusion of thrombogenic material into the vessel lumen and acute thrombosis[14]. Modest elevations of systemic measures of inflammation – such as C-reactive protein (CRP), interleukin-6 (IL-6) and soluble vascular endothelial molecules – are independent predictors of future CV events in the general population[15-17].

RA is a chronic inflammatory disease characterized by high serum levels of the same inflammatory cytokines that have been implicated in the pathogenesis and rupture of atherosclerotic plaque (e.g. IL-1, TNF-α, IL-6)[14]. This highly inflammatory milieu is hypothesized to accelerate the atherosclerotic process in patients with RA. Attempts to prove this hypothesis have predominantly correlated single measures of inflammation with CV events, but only one CRP study approached statistical significance (HR 1.10; 95% CI 0.99-1.22)[18], and two sedimentation rate (ESR) studies showed correlation with CVD[18,19]. Other studies have correlated clinical indices of RA disease activity and RA-associated autoantibodies with atherosclerotic plaque as well as incident CV events[20-22]. *These prior study approaches were indirect, non-experimental and often retrospective*.

With the advent of PET scanning and the development of radioisotopes that are avidly taken up by monocytes/macrophages (e.g., FDG), the ability to detect inflammation within vessel walls and specifically within atherosclerotic plaque has been realized[23]**.** *In this proposal, we will - for the first time in the setting of an RCT in RA – directly assess inflammation in the vascular walls of patients with RA, using vascular FDG PET/CT imaging.* We will also correlate DMARD associated changes in vascular inflammation with changes in RA disease activity as measured by clinical (DAS28), biomarker (MBDA) and joint imaging (FDG PET/CT) measures, as well as separately with changes in systemic inflammation as measured by CRP and IL-6 levels.

2.1.3 Currently available CV risk tools underperform in identifying CV risk in RA .

In the general population, CVD risk prediction tools (e.g., Framingham, FRS,[24] and Reynolds, RRS[25]) have been developed to estimate an individual's future risk for a CV event. These risk tools perform well at the population level, but they do not discriminate risk well in women and younger individuals[26], groups heavily represented in RA. In fact, Crowson et al[27] reported that the observed CVD risk in 525 RA was 2-fold higher in women and 65% higher in men than the FRS predicted risk, with similar findings from the RRS despite its inclusion of CRP. CV risk in RA may be associated with lower, not higher, levels of total and LDL cholesterol, further illustrating the problems with conventional CV risk measures in stratifying CV risk in RA[18,28-30]. A CV risk assessment tool has been endorsed by the European League Against Rheumatology (EULAR), but its evidence base is weak and its performance poor[31]. *These deficiencies of currently available CV risk tools in RA populations underscore the pressing need for identification of biomarkers of CV risk tailored for the RA patient.*

Prior efforts aimed at developing risk scores in the general population have used imaging biomarkers, coronary artery calcium and carotid intima medial thickness (cIMT). However, a recent meta-analysis of prospective RCTs suggested that cIMT changes did not correlate well with rates of future clinical CVD[32]. In contrast, FDG PET/CT is being increasingly used in RCTs because inflammation within vascular walls and within plaque is believed to represent one of the critical early and reversible steps of atherosclerosis[33,34]. In fact, several recent studies have demonstrated that the presence of arterial inflammation (measured by FDG uptake) identified individuals at higher risk for future CV events than those without inflammation[35,36].

2.1.4 FDG PET/CT is a sensitive method for detecting vascular inflammation

Although most widely used for detection of cancer, FDG PET/CT imaging represents a novel modality for direct detection of inflammation in the vasculature. Cellular FDG uptake reflects the rate of tissue glycolysis, which is higher in areas containing inflamed tissues. Activated macrophages have remarkably elevated glycolytic flux [37,38] and hence avidly accumulate FDG [39]. Moreover, FDG uptake in atherosclerotic plaques correlates with the density of macrophages determined histologically [23,40-43]. Indeed, FDG PET/CT is now used clinically to evaluate inflamed tissues, and has recently been the focus of guidelines from American and European Societies for Nuclear Medicine and Molecular Imaging[44].

FDG PET/CT for Imaging Atherosclerosis: In atherosclerosis, disease activity can be defined as progression of atherosclerotic plaque or development of atherothrombotic events. In a multi-center trial employing both PET/CT and MRI imaging, changes in the PET/CT signal predicted the rate of plaque expansion eighteen months later[45]. In another study, the arterial PET signal predicted the subsequent rate of atherosclerotic plaque progression, based on CT imaging[46]. Additionally, several studies have shown an association between the FDG signal and CV risk factors or risk scores[35,47-49]. Higher arterial FDG uptake was also associated with a substantially increased risk for subsequent stroke and MI[36,50]. Thus, there is substantial evidence to support the utility of FDG PET/CT imaging to predict atherosclerotic disease progression and subsequent CV events. This imaging modality is responsive to changes to CV risk as a result of treatment. High dose statin therapy reduced FDG PET/CT signal to a greater extent than low dose statins[51,52]; clinical outcome studies have found a 28% reduction in CV events among high dose statin users versus low dose[53].

FDG PET/CT as a Tool to Measure Changes in Tissue Inflammation after Interventions: FDG PET/CT imaging is a well-established tool for evaluating treatment-related changes in inflammation after interventions in humans. Several groups have shown that statin therapy (a well-described anti-inflammatory treatment in atherosclerosis) results in a significant reduction in arterial FDG uptake after 3 months[52,54,55]. Using FDG PET/CT, Tawakol (co-I) et al observed a relatively rapid reduction in arterial inflammation in individuals randomized to atorvastatin 80 (compared to atorvastatin 10mg)[52]. More recently, van Wijk et al[56,57] demonstrated in individuals with familial hypercholesterolemia that non-pharmacologic lipid lowering by Lipoprotein Apheresis induces a rapid reduction in arterial inflammation within one week of the intervention. Additionally, FDG PET/CT imaging has been used to study the impact on arterial inflammation of therapies that do not substantially impact serum LDL. Recently completed multi-center PET/CT imaging trials evaluated the anti-inflammatory efficacy of p38 MAP kinase antagonism[58], a novel innate-immune modulator lecinoxoid[59], Lipoprotein-associated phospholipase A2 (Lp-PLA2) antagonist[60], and a cholesteryl ester transfer protein (CETP) antagonist[61]. Others have reported on the impact of tumor necrosis factor antagonists[62] and pioglitazone [63].

Relationship between FDG PET/CT Imaging Trial Findings and Clinical Endpoint Trial Results: Currently, results of both clinical endpoint studies and FDG PET/CT imaging trials are available for 4 drug classes. Such paired results (albeit from different RCTs) allow the evaluation of the degree to which FDG PET/CT trial results are predictive of clinical endpoint studies. The 4 drug classes are 1) statins, 2) pioglitazone, 3) CETP antagonist, and 4) Lp-PLA2 antagonists. As previously stated, each of the published PET/CT studies evaluating the impact of statins on the vasculature demonstrate a reduction in atherosclerotic inflammation, hence those findings parallel the well-described reduction in CVD events associated with statins. Similarly, pioglitazone is associated with both a reduction in arterial FDG[63] and a reduced incidence of atherothrombotic events.[64,65] Hence for statins and pioglitazone, findings of reduced arterial inflammation in FDG PET/CT imaging trials are consistent with the findings of clinical endpoint trials. On the other hand, the dal-PLAQUE imaging trial which evaluated the effect of dalcetrapib (a CETP modulator) on arterial plaque showed no difference between dalcetrapib and placebo (p=0.51) on the primary or secondary FDG PET/CT imaging end-point [66]. The subsequently published dal-OUTCOMES trial evaluating the effect of dalcetrapib on major coronary events similarly demonstrated no clinical benefit.[67] Likewise, an FDG PET/CT imaging trial evaluating an inhibitor of Lp-PLA_2, rilapladib, showed no beneficial impact of Lp-PLA_2 antagonism on arterial inflammation[60]. Subsequently, the STABILITY trial of nearly 16,000 individuals showed no beneficial effect of darapladib, also a Lp-PLA2 antagonist, to reduce the primary composite endpoint of CV death, MI, or stroke.[68] *Thus for all 4 drug classes for which there are both FDG PET/CT data and clinical endpoint data (atorvastatin, darapladib/rilapladib, dalcetrapib, and pioglitazone), the imaging trial findings have been consistent with the results from clinical endpoint studies*. While the FDA has not yet accepted FDG PET/CT as a validated surrogate measure of CVD, the use of FDG PET/CT imaging may provide a pathway for predicting the potential efficacy of CV therapeutics

using a relatively small number of patients and with short observation periods of 1 to 3 months. The current trial may provide supportive information to move forward with an outcomes trial.

Arterial FDG PET/CT studies in RA. Recently, Maki-Petaja et al[62] reported higher aortic FDG uptake in 17 RA patients compared to 34 non-RA controls with stable CAD (target-to-background ratio [TBR] 2.02±0.22 vs 1.74±0.22, respectively, p=0.0001). Moreover, open label treatment of the RA patients with a TNFi (etanercept or adalimumab) over only 8 weeks was associated with an 18% decrease in FDG uptake (2.51±0.33 to 2.05±0.33, p<0.0001 in the most diseased segment) that correlated with decreases in CRP and DAS28 levels. A cross-sectional study of 10 psoriasis and 5 RA patients also demonstrated higher FDG uptake at all levels of the aorta compared to uptake in 10 healthy subjects after adjusting for conventional CV risk factors[69]. These data are limited by very small sample sizes and absence of RA treatment placebo or comparator groups. However, they do suggest the potential value of FDG PET/CT to assess vascular inflammation in RA. We have conducted a retrospective study of 33 RA patients who underwent PET/CT imaging for non-CVD indications (in press), and a cross-sectional observational study of 71 RA patients who have undergone aortic PET/CT imaging. *Taken together, these studies strongly support the feasibility of enrolling adequate numbers of RA patients with elevated vascular FDG uptake for the purposes of the proposed RCT.*

2.1.5 The effect of RA treatments on CVD is unclear.

To date, there is no level A evidence (i.e., double blind RCTs) that any RA DMARDs reduces frequency of CV events in patients with RA. However, there is a strong body of experimental data that lay the groundwork for this hypothesis, *and these data are by far most robust for the TNFi's at every level (in vitro, animal models, human observational data)*.

TNFα and TNFi effects on vasculature. Vascular endothelium is a select target for TNFα in which it induces an array of pro-inflammatory, pro-coagulant, and pro-apoptotic genes that damage endothelium and increases thrombotic potential, while simultaneously inhibiting synthesis of the vasodilator nitric oxide[70]. TNFα also induces endothelial adhesion molecules which mediate attachment and transmigration of inflammatory cells to the arterial walls[71]. The monocyte influx takes up oxidized LDL, activating release of TNFα and further promoting LDL uptake via induction of acyl-CoA-cholesterol transferase 1[72]. This feed forward loop results in foam cell accumulation, an early stage of atherosclerosis[72]. These effects can be reversed by TNFi's in vitro[73]. Mice deficient for both TNFα and apoE exhibit reduced atherosclerotic lesions compared to wild type controls[74]. Therapy with TNFi's as an anti-atherogenic strategy is strongly supported by experimental data.

Given these data and the observation by Feldman and Maini that TNFα is also the primary cytokine driving joint and systemic inflammation in RA[75], there has been intense interest in investigating inhibition of TNF as a strategy in RA for reducing CVD. In small studies of RA patients, the prevalence of endothelial dysfunction and aortic stiffness were observed to be higher than in controls. TNFi treatment transiently and partially reversed these measures[76,77] At the macrovascular level, several cohort studies suggested that TNFi's also slow progression of asymptomatic carotid atherosclerosis compared to conventional DMARDs[78,79]. As to whether TNFi's reduced CV events in cohort-based epidemiological studies in RA, the data are somewhat more mixed. For example, several large registry studies showed a reduction in CV events in TNFi users compared to non-users[80,81], but studies from other large databases such as the US VA health system and the Swedish and British Rheumatology Registries[82-84] did not demonstrate reductions in rates of composite CVD endpoints with exposure to TNFi's. Many of these registry and administrative data studies lack detailed information on traditional CV risk factors and RA characteristics. Thus, confounding by indication and other unmeasured confounders threaten their validity, severely limiting the causal inference. *The time is right therefore to move the field forward in RA via an RCT to directly evaluate the effect of*

TNF inhibition on a vascular measure (i.e., arterial inflammation) that is an integral feature of atherosclerosis and plaque rupture.

Effect of non-biologic DMARDs on the vasculature. In contrast to the robust data suggesting a therapeutic role for TNFi's in treating atherosclerosis, data for SSZ and HCQ are extremely limited. Despite decades of SSZ use as a treatment for RA, and anti-inflammatory actions that have potential relevance to atherosclerosis (e.g., inhibition of activation of NFkB and scavenging of reactive oxygen species[85,86]), there is almost no information regarding a potential effect on reducing CVD. A small RCT in non-RA patients with stable CAD evaluated the efficacy of SSZ vs placebo for improving endothelial function[87] but no significant difference was observed. In a large multinational RA cohort in Europe, comparing users of various DMARDs to non-users, SSZ was associated with only a modest reduction in CV morbidity (HR 0.92, 95% CI 0.87-98) compared to MTX (HR 0.85), leflunomide (HR 0.59), glucocorticoids (HR 0.95) and TNFi's (HR 0.42)[88].

HCQ is an anti-malarial drug with modest anti-inflammatory effects in RA but a disease modifying capacity has never been definitively confirmed. It has effects on both the adaptive as well as innate immune pathways. HCQ raises the intracellular pH of lysozomes which impairs protein processing, causing downstream impairment of immune functions such as antigen presentation to CD4+ cells, interaction of intracellular toll-like receptors (TLRs) with cognate ligands, and production of proinflammatory cytokines[89-91]. These mechanisms could play a role in treating/preventing atherosclerosis but there are no data to support this at present. Interesting data do support an effect of HCQ in reducing metabolic risk. Gu et al[92] showed that anti-malarials suppress lipid accumulation in macrophages through their effect on TLR-9. However, in a recent meta-analysis of nine studies in SLE, evidence suggesting that anti-malarials reduce lipid levels was weak[93] although another study reported a protective effect in SLE against metabolic syndrome[94]. In two RA cohort studies, treatment with HCQ was associated with a decrease in incident diabetes[95,96], while a third study did not show a significant reduction[30]. There are no data on the effect of HCQ in reducing CV events.

Taken together, *these data strongly support our hypothesis that TNFi therapy will have a more potent effect on arterial inflammation than combined SSZ+HCQ (with both given on MTX background).* While our study design does not allow us to investigate the effect of MTX on arterial inflammation, our preliminary data and that of Maki-Petaja[62] strongly suggest that MTX-IRs will have more than sufficient arterial inflammation to measure a drug effect.

2.1.6 Blood-derived biomarkers of CV risk in RA are also needed.

Blood-derived biomarkers of CV risk (e.g., SNPs, gene arrays, soluble proteins, cell subtypes) are also desirable and a focus of great interest. Advantages of blood-derived biomarkers of CV risk include: 1) easy accessibility, 2) lack of radiation exposure, and 3) ability to standardize assays that enable widespread commercial use. As with imaging biomarkers, blood-derived biomarkers would ideally identify asymptomatic RA patients at intermediate to high CV risk who would not be identified by current conventional CV risk models such as the FRS. *This would enable a risk-stratified approach combining conventional CV risk factors and RA-specific factors which could substantially reduce CV associated morbidity and mortality in patients with RA.*

Correlations of a single non-specific biomarker of inflammation (e.g., CRP, sedimentation rate [ESR] or IL-6) with CVD in patients with RA have been modest[97-99]. Similarly, clinical indices of RA disease activity, and RA-associated autoantibodies, have met with similarly limited success as 'biomarkers' of CVD[20-22]. A broad array of biomarkers reflecting systemic inflammation, endothelial activation, lipid metabolism and pro-inflammatory cytokines have been shown in the general population to be associated with prevalent and/or incident CVD. These include CRP, IL-6, serum amyloid protein (SAA), vascular cell adhesion molecule (VCAM), apolipoprotein B and others[100]. *A substantial number of these CV risk biomarkers also reflect biological pathways that are activated in RA and*

correlate with RA disease activity. For example, of the twelve proteins that comprise a commercially available MBDA known as 'Vectra-DA' (Crescendo Bioscience), all are also recognized as CVD biomarkers, such as CRP, SAA, IL-6, TNF-R1, MMP-1 and -3, leptin, resistin, VCAM, vascular endothelial growth factor-A, epidermal growth factor, and human cartilage glycoprotein-39 (YKL-40)[101] *Thus, the MBDA is a very promising biomarker for investigation of CV risk in patients with RA.* It was derived from studies of 130 biomarkers; of these, 25 were selected for algorithm training, and the final MBDA consisted of the 12 biomarkers noted above[102]. The MBDA has been found to have strong correlation with the DAS28-CRP (r^2=0.60), significantly better than the CRP (r^2=0.38)[102]. In an Area Under the Receiver Operating Curve (AUROC) analysis examining the ability of the MBDA to classify patients into low disease activity vs moderate-high disease, the average AUROC was 0.89[102]. MBDA scores range between 1 and 100. Performance characteristics of the MBDA have been confirmed in independent cohorts, and with additional validated RA disease activity measures including the SDAI, CDAI and RAPID3[103]. Finally, data suggest that the MBDA 'molecular remission' may be superior to clinically defined remission in predicting progression of radiographic joint damage[104]. *If the MBDA is also found to predict change in vascular inflammation in RA, this could enhance current CVD risk models in RA with potential clinical value*.

2.1.7 FDG PET/CT can also be used to assess treatment-associated changes in joint inflammation.

FDG is taken up by inflamed joints, and several small pilot studies assessing disease activity in RA have shown promising correlations between FDG uptake and disease activity. Palmer et al[105] found excellent anatomical correlation in the wrists of 12 RA patients between region of greatest FDG uptake with volume of pannus determined by MRI sequences. Beckers et al[106] found a strong association between FDG uptake in 356 joints of 21 RA patients with clinical and ultrasound derived measures of joint activity. In a further study by the same investigators of 16 RA patients treated with a TNF antagonist[107], reduction in FDG uptake in the knees at four weeks was highly correlated with changes observed in all MRI parameters and with MMP-3 levels, but less well with serum CRP levels. Okamura et al [108] also studied change in FDG uptake in 22 RA patients treated for 6 months with a TNFi; average decrease in maximal standardized uptake value (SUV_{max}) after six months was 22% and was positively correlated with change in DAS28-CRP levels (r=0.66, p=0.001). Roivainen et al[109] examined change in FDG uptake after initiation of triple therapy plus low dose prednisone in 17 patients with RA. 76% and 81% of patients showed reductions in SUV_{max} at weeks 2 and 4, respectively; mean decreases in SUV_{max} were 22±13% and 29±13%, respectively. While encouraging, these studies were extremely small and open label. *In the proposed study, we will for the first time utilize FDG PET/CT in an RCT to evaluate change in joint inflammation between two treatment regimens for RA.*

2.1.8 Bioethics Ancillary Study

There is a lack of information available on researchers' attitudes and beliefs regarding incidental research findings and the prevalence of incidental research findings from state of the art imaging studies, such as whole body FDG PET/CT in those without known malignancy, and to our knowledge, no such information available in rheumatology. The Treatments Against RA (Rheumatoid Arthritis) and Effect on FDG PET/CT (TARGET) study provides an unprecedented and unique opportunity to examine the ethics around incidental findings from whole body FDG PET/CT in RA patients without known malignancy.

2.1.9 Neuroimaging Measures Ancillary Study

Neuroimaging has revolutionized the study of neuropsychiatric conditions, including conditions along the stress axis (anxiety, stress, PTSD, and depression). Imaging with functional MRI and FDG PET provide insights into regional reactivity (fMRI) or resting metabolism (FDG PET) of regions of the brain that are critically involved in stress conditions (including the prefrontal cortex and amygdala). FDG-PET assessments of regional brain activity has been performed for decades, and leverages the fact that FDG localizes to areas of active glycolysis. This signal is

reproducibly quantified using PET images that are co-registered to CT for anatomical localization. Higher rAmygA may be a more accurate reflection of chronic stress, higher trait-anxiety, and persistent negative affect[110], and has repeatedly been shown to associate with anxious temperament[111] perceived stress[112], and PTSD[113]. Furthermore, this signal is physiologically important, as it links to diseases that are independently associated with stress, such as cardiovascular disease[112]. Moreover, higher rAmygA was shown to link to future CVD events through upregulated activity in bone marrow and heightened arterial inflammation in series.

Rheumatoid Arthritis is associated with an Elevated Chronic Stress Burden, and an Exaggerated Stress Response. RA patients commonly report that disease flares occur during or after periods of increased psychological stress[114]. However, studies attempting to causally link stress with RA disease activity and/or systemic inflammation have been inconsistent[115], hampered by using self-reported measures of psychosocial stress, difficulty establishing temporality, and the inability to evaluate the contribution of RA symptoms, pain, and disability to stress. Few studies in RA have utilized neuroimaging to investigate links between the brain, immune system, and joints, and no studies have explored the links between neuroimaged reactivity in the amygdala and related brain structures and vascular inflammation in RA patients, despite compelling associations between self-reported psychosocial stress and coronary calcification in this population. Establishing these associations will inform mechanistic follow-up studies, validate the performance of self-reported psychosocial stressors as proxy measures, and support the testable concept of psychosocial stress management as an intervention for reducing CVD and disease activity in RA.

2.2 Rationale

We considered several alternative designs. We first considered a *clinical outcomes trial* with CV events as the endpoint. This design was part of our original NIH planning grant application, and it was rejected by the Study Section because of feasibility. While an outcomes trial would be most clinically relevant, we have come to agree that it is not feasible. Based on an assumed 25% difference in CV event rates and a 2% annual event rate, we calculated a sample size of ~10,000 followed for a median of 3 years. Maintaining the original treatment arms in an RA trial for 3 years is impractical. The total cost would be approximately $70M. If we could enroll a very high-risk RA population (e.g., immediately post-MI), then fewer subjects would be required. However, since IL-6 blockers and JAK inhibitors raise LDL, the ethics of this trial are unclear. Thus, a surrogate endpoint trial with FDG PET/CT is the preferred design.

We considered several *potential treatment comparisons*. We pursued designing a treat to target (TTT) trial with the Technical Expert Panel (TEP) through a formal Delphi Panel process. This involved extensive literature reviews, protocol development, and consensus voting on 10 key aspects of the protocol. This process is outlined in a submitted manuscript[110]. While we reached consensus on all issues, there were a multitude of operational issues raised by the TEP. Several of the most difficult issues include: how to ensure that usual care would differ substantially from TTT; the clinically appropriate drug dosing and visit regimens for TTT that would facilitate separation of the disease activity curves for TTT versus usual care; the use of glucocorticoids in the TTT regimen, and the strong potential for contamination across arms. After considering these issues, we determined that it would be extremely difficult to conduct a rigorous TTT trial focused on the CV endpoint described above. We considered several comparisons for a treatment trial.

We *chose to pursue a TNFi + MTX versus triple therapy for several reasons.* 1) While there are strong data to suggest that these treatments produce similar clinical outcomes by 12-24 months[111-113], triple therapy is a strategy that is not being widely used in typical practice. Thus, further information comparing these strategies may help clinicians and patients make treatment decisions. 2) TNFi + MTX appears to have a quicker onset of action with greater disease control at 6 months based on several published trials[111,113]. Thus, there is ample evidence to support a superiority hypothesis for the proposed trial. 3) Both treatment options are considered standard of care, enhancing patient and provider acceptability. 4) The ability to adjust doses over the 6-month trial for both regimens will facilitate patient retention. The doses of MTX, SSZ and several TNFi can be titrated.

Finally, we considered a longer trial originally with the desire to provide information over a longer treatment course. However, there are two critical problems with a longer trial. First, as noted above, some patients will not respond to treatment regimens. We believe that it is ethical and feasible to keep patients on dose-adjusted treatments for 6 months, but not longer. Second, 6 months will allow for improvement in FDG PET/CT. This has been demonstrated in prior trials using statins with an FDG PET/CT assessment after 12 weeks[52,114].

3. STUDY DESIGN

3.1 Overview

This is a multi-center, phase IV, 24-week, two arm randomized clinical trial to evaluate the effects of DMARD associated changes in vascular inflammation in patients with RA. The overall study design is shown in Figure 1. Two hundred patients with RA who are inadequate responders to MTX (MTX-IRs, DAS28>3.2) will be randomized in a 1:1 ratio to receive either a TNFi or SSZ + HCQ, while continuing background MTX (and HCQ if applicable), for 24 weeks. Although the joint count assessors and imaging readers will be blinded to treatment assessment, the subjects and investigators will not be blinded.

3.2 Study Population

This will be a multicenter study with approximately 40 study sites. 400 RA patients will be recruited over a planned recruitment period of 24 month, 200 will be randomized. The target population consists of patients who are deemed methotrexate-inadequate responders (MTX-IRs, DAS28>3.2) by their treating rheumatologist, and who have not yet stepped up to additional treatment with a biologic DMARD.

Patients will be identified for potential recruitment by methods that may include using physician referral, pre-screening enrollment logs, IRB or Sponsor approved sources, such as newspaper/radio advertisements and/or mailing lists. Informed consent will be obtained by the Principal Investigator, co-investigator, or Study Coordinator at each site. Study procedures including screening procedures will not begin until signed informed consent has been obtained.

3.3 Inclusion Criteria

Subjects who meet all of the following criteria at Screening are eligible for enrollment into the study:

- Written informed consent signed by the subject;
- Fulfill ACR/EULAR 2010 criteria for RA;
- Men ≥ 45 years and women ≥ 50 years;
- MTX for ≥ 8 weeks at ≥ 15mg weekly or on at least 7.5mg of methotrexate weekly for ≥8 weeks with a documented intolerance of higher MTX doses, and on a stable dose for the previous 4 weeks;
- DAS28 score > 3.2;
- Able to swallow pills;
- Males and females with reproductive potential must agree to practice effective measures of birth control;
- If taking prednisone (or equivalent corticosteroid), the dose must be ≤ 10 mg/day at the time of the baseline FDG PET/CT scan and must NOT change by more than ±3.0 mg for the four weeks prior to the baseline FDG PET/CT; (please see Corticosteroids Section 4.4.1 for further details);
- If taking a low- or moderate-intensity statin, the dose must be stable for six weeks prior to screening and must not change during the six months of the trial (please see Statins and PCSK9 Inhibitors Section 4.4.2 for further details);
- Willing to comply with all study procedures and be available for the duration of the study;
- Rheumatoid arthritis diagnosis without psoriasis or with psoriasis if rheumatoid factor ≥ 2x ULN or anti-CCP ≥ 2x ULN.

3.4 Exclusion Criteria

- Prior use of biologic DMARD or small molecule DMARD (i.e. tofacitinib) in the past 6 months, use of Rituximab ever;
- If a subject is considered to be an etanercept (Enbrel) or adalimumab (Humira) failure by their primary rheumatologist;
- Non-biologic DMARDs other than MTX or HCQ for two months prior to Screening;
- Current use or use within the past 12 months of a high-intensity statin lipid lowering drug (atorvastatin/Lipitor 40mg or higher, rosuvastatin/Crestor 10mg or higher) or a PCSK9 inhibitor (alirocumab/Praluent, Evolocumab/Repatha, or Bococizumab);
- Prior patient reported, physician diagnosed clinical CV event: myocardial infarction or heart attack, angina, stroke, uncompensated or severe heart failure (NYHA class III or IV), prior vascular procedure (coronary artery angioplasty or stenting, carotid endarterectomy, coronary artery bypass surgery);
- Demyelinating disease;
- Any of the following forms of arthritis that may otherwise explain the subject's RA symptoms: Psoriatic Arthritis, Reactive Arthritis, Juvenile Idiopathic Arthritis, Ankylosing Spondylitis, Polymyalgia Rheumatica
- Any of the following other autoimmune and/or chronic inflammatory diseases: Inflammatory Bowel Disease, Crohn's disease, Cutaneous or Systemic Lupus, Systemic
  Vasculitis, Giant Cell Arteritis, Polymyositis, Dermatomyositis, Sarcoidosis, or Scleroderma;
- Transient ischemic attack (TIA);
- Revascularization for peripheral artery disease;
- Cancer treated in last five years (except basal and squamous cell) or any lymphoma or melanoma;
- Type I diabetes mellitus or type II diabetes mellitus treated with insulin or uncontrolled with HbA1c ≥7%;
- Known pregnancy, HIV, hepatitis B, hepatitis C, active (or untreated latent) TB;
- Known sulfa allergy or other known hypersensitivity to any of the trial agents or G6PD deficiency;
- Known macular disease or known retinal disease;
- Baseline blood count, renal or liver abnormalities as follows: WBC < 3.5 x 1000 n/ul, Hematocrit < 30%, Platelet count < 90 x 1,000 n/ul, estimated glomerular filtration rate < 50 ml/min/1.7m^2, AST >60 U/L, ALT>84 U/L;
- Intra-articular injection within the 4 weeks prior to the potential baseline FDG PET/CT; and
- Two or more of the following high dose radiation scans in the past year: CT scan with contrast, angiogram, SPECT nuclear medicine scan, myocardial (cardiac) perfusion scan.

3.5 Recruitment and Retention

3.5.1 Recruitment Plan

The target population to be recruited is men aged 45 and older or women aged 50 and older with a clinical diagnosis of RA who are deemed methotrexate-inadequate responders (MTX-IR) by their treating rheumatologist. Patients will be recruited from participating rheumatology and arthritis clinics and practices at participating sites across the United States. A recommended method for identifying and recruiting patients is described in the following sections and in more detail in the MOOP. Sites will generate and maintain a list of patients that may become eligible for the study based on a HIPAA-compliant review of medical records of existing RA patients. The treating rheumatologists will alert study staff when a patient is deemed a MTX-IR, prior to a switch or escalation of DMARDs. The Site Coordinator will speak with the patient to go over pre-screening inclusion/exclusion criteria, explain the trial, and assess interest (Recruitment training will be provided). The Study Brochure can be given to interested patients.

If the patient is interested in participating, a Site PI, Co-I, or Site Coordinator must obtain informed consent prior to any screening procedures. The research protocol will be explained in detail, including all possible risks and benefits. During the consent process, the study coordinator or investigator probes the patient's understanding of the

protocol. All participants will be told that the study is voluntary, that they have no obligation to participate, and that they may decline or withdraw at any time without compromising their care. Subjects will have ample opportunity to ask questions prior to signing the consent form. Eligible subjects will be allowed as much time as necessary to determine whether they wish to participate in the study.

3.5.2 Schedule of Study Events

Consent will be obtained at the beginning of the Screening Visit (Visit 1). Patients who meet eligibility criteria and provide informed consent will be considered enrolled and will undergo the additional screening procedures outlined above in Table 1, including completion of questionnaires, screening laboratory tests including hepatitis B and C screens and tuberculosis screening (if none in the past six months),hemoglobin A1c test only for Type II diabetics if none from the past six months, and chest x-ray without evidence of interstitial lung disease (if none in past 12 months). Subjects found at Screening to have active hepatitis or active infection (including tuberculosis) or interstitial lung disease will be excluded. If the additional required screening blood work was completed by the subject within 1 week of the screening visit (see 3.4 above), these tests do not need to be repeated. If the subject has been on stable methotrexate or prednisone for the 8 weeks preceding the screening visit, then safety labs performed during those 8 weeks do not need to be repeated at the screening visit. CRP testing must be completed at the date of Screening when a joint count is done to determine the subject's eligibility for study inclusion. If these additional laboratory- and imaging-based eligibility criteria are met and eligibility is confirmed, the FDG PET/CT scan will be scheduled. For the FDG PET/CT, the subject will be given instructions for an overnight fast for the purposes of the PET/CT scan. If nothing is found on the FDG PET/CT that would preclude the subject from randomization, the subject will be randomized and a baseline visit will be conducted. The subject will complete additional questionnaires at baseline. All subsequent study visits are performed at six week intervals and should be conducted within 14 days prior to or after the designated date (calculated from the subject's randomization date). Blood will be drawn at each visit for safety monitoring; additional fasting blood specimens will be drawn at Visits 2, 3, 5 and 6 for the research biospecimen repository. Safety labs may be deferred in extreme circumstances (i.e. COVID-19 pandemic). During this period when patients and clinicians are trying to minimize contact with the health care system, we will rely on site PIs' clinical judgement for the appropriate frequency of safety laboratory testing. However, this should be no less than every six months and no greater than every six weeks. A repeat FDG PET-CT scan will be performed between 24 and 36 weeks.

3.5.3 Randomization and Blinding

Upon confirmation of subject eligibility, the Site Coordinator will determine treatment assignment through a randomization function of the electronic data capture (EDC). Randomization will be performed centrally by the DCC using a permuted block design. Metrologists performing joint counts will be blinded to treatment assignment, as will the FDG-PET/CT imaging readers. Laboratory staff who run bioassays on biospecimen samples will also be blinded to treatment assignment. Subjects will be instructed NOT to discuss their treatment with the metrologist, and the metrologist will not be a part of the subject's care team. The metrologist will also be instructed to remind the subject before each joint examination that treatment should not be discussed. In addition, the metrologist should not discuss treatment with the site PI or other study staff.

3.6 Subject Retention

We aim to have a 90% retention rate in order to meet the guidelines established by the NIH. Once enrolled, a proactive plan for retention will be implemented that includes elements such as regular phone and mailed reminders for each study visit, parking and meal vouchers, complimentary items such as pens and canvas totes with the study logo, birthday and holiday cards, etc. Arguably, most importantly, we will train sites to follow major principles and commonly used strategies to maximize retention and minimize loss to follow-up. These are outlined in detail in the MOOP and include

building subject relations and subject satisfaction, with the study coordinator taking a central role in this effort, emphasizing the importance of congeniality, respectfulness and friendliness in interactions with participants.

3.7 Subject Withdrawal

All subjects receive active therapy and the trial is relatively short in duration; thus, we anticipate few withdrawals and/or cross-overs. Furthermore, the ability to titrate the regimens will limit withdrawal and/or cross-over. As noted above, great effort will be made to retain subjects in the study. Nonetheless, subjects have the right to refuse treatment or completely withdraw from the study at any time for any reason. An explanation of why the subject is withdrawing from the study should be recorded on the withdrawal eCRF. The investigator also has the right to withdraw subjects from the study treatment in the event of AE, protocol violations, administrative reasons, or for other reasons. When applicable, subjects should be informed of circumstances under which their participation may be terminated by the investigator without the subject's consent. Any administrative or other reasons for withdrawal must be documented and explained to the subject. If the reason for removal of a subject from the study is an adverse event, the principal specific event will be recorded on the CRF. The subject should be followed until the AE has resolved. If it appears that a subject is lost to follow-up, the investigator must attempt to contact the subject or a responsible relative by telephone, to determine if any new AEs occurred, follow-up of any ongoing AE, and to establish as completely as possible the reason for the withdrawal.

Study treatment may be discontinued for any subject who experiences any of the following:

- Extreme laboratory value (see **Section 4.5.1 Abnormal Lab Values**)
- Malignancy other than basal or squamous cell
- Repeated subject non-compliance or loss to follow-up.
- The subject withdraws consent
- The investigator or DSMB believes it is in the best interest of the subject
- The study is terminated

If study treatment is discontinued (e.g., for an adverse event) and there is no safety issue precluding it, the subject will be asked to return for the follow-up FDG PET/CT scan if he/she has received at least eight weeks of the randomized treatment prior to withdrawal.

In addition, if the subject decides to withdraw from treatment assignment before trial completion, the subject will be asked to come in for a follow-up FDG PET/CT scan within 2 weeks of stopping their randomized treatment assignment and before initiating any new treatment. Subjects will only be asked to come for a follow-up FDG PET/CT if they have been on the trial assigned treatment for at least 8 weeks. If they have been on less than 8 weeks of study treatment before wanting to withdraw, they will not undergo a second scan.

If the subject withdraws consent, all study related visits, exams, procedures and data collection are terminated.

3.8 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated by the DSMB or by the NIH if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to investigators, funding agency and regulatory authorities. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

3.9 Bioethics Ancillary Study

We will survey the TARGET investigators to determine researchers' attitudes and beliefs regarding their ethical obligation to return and manage incidental research findings from whole body PET/CT imaging studies and determine the prevalence of incidental extra cardiac findings in whole body PET/CT scans in rheumatoid arthritis (RA) and detection rate of previously unknown malignancies.

Dr. Kang, a bioethicist with training in survey methods, will develop the survey. The survey design will be created and informed using the existing literature, previously used surveys on incidental findings from research and in consultation with advisors who are Columbia University faculty members with extensive experience with survey methods. The survey would include Likert scale, forced choice, rank order, and open-ended questions. The open-ended questions will provide participants an opportunity to provide additional information that may not be adequately captured in the other components of the survey, and will also provide qualitative data.

Once developed, the survey would be entered into REDCap (Research Electronic Data Capture) for distribution. Responses will be collected anonymously via REDCap. All surveys will initially be distributed through email, which includes a link to the electronic version of the survey. If there is no response to an initial email, the participants will receive a total of 3 email reminders. To increase the response rate, if there is no response via email, a paper copy of the survey will be mailed to participants. Site PIs will be compensated for their participation in the proposed survey.

4. TREATMENT AND STUDY PROCEDURES

4.1 Acquisition of Randomized Treatment

All study medications will be provided to participating subjects excluding methotrexate. As participants entering the study are already taking methotrexate and will be continuing to take that medication for the duration of the study, subjects will continue to obtain their methotrexate through their previously utilized source.

Upon completion of screening procedures and determination that the subject is eligible for inclusion in the trial, the subject will be randomized to trial treatment through the Data Coordinating Center. Once a subject has been assigned to treatment, the coordinating center will be alerted. The coordinating center will then immediately notify the drug distribution center of the appropriate treatment that must be sent out. A drug kit will be sent from the drug distribution center to the site. The subject will then return to the study site to pick-up the drug and complete a baseline visit. Subjects will begin their treatment arm on the date of receipt of the medications.

4.2 Description of Treatment Medications

A brief description of each of the study medications (all of which, as noted above, are FDA approved) is provided below. More detailed descriptions of each drug are provided in the following links to the FDA approved package inserts:

1) Adalimumab: http://www.rxabbvie.com/pdf/humira.pdf
2) Etanercept: http://pi.amgen.com/united_states/enbrel/derm/enbrel_pi.pdf
3) Sulfasalazine: http://labeling.pfizer.com/ShowLabeling.aspx?id=524
4) Hydroxychloroquine: http://www.accessdata.fda.gov/drugsatfda_docs/label/2007/009768s041lbl.pdf
5) Leflunomide: http://products.sanofi.us/arava/arava.html
6) Methotrexate Injection: http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/011719s117lbl.pdf
7) Methotrexate tablets: http://www.rheumatrex.info/pdf/RheumatrexPackageInsert.pdf

Etanercept. The standard dose of etanercept is 50 mg SC once weekly. The drug is provided already diluted to the appropriate concentration in pre-filled syringes. It is typically injected into the abdomen or thigh. The medication must be refrigerated.

Adalimumab. The standard initial dose of adalimumab is 40 mg SC once every other week. Like etanercept, it is provided already diluted to the appropriate concentration in pre-filled syringes. It is also injected into the abdomen or thigh and must be refrigerated.

Sulfasalazine. SSZ is formulated as tablets for oral use. Maximum dosing is usually 1500 mg bid. Patients with Sulfa allergies or G6PD deficiency should not be prescribed this medication and are excluded from the study.

Hydroxychloroquine. The approved dose of HCQ is either 200 mg once daily or twice daily. HCQ dose shall not exceed 6.5 mg/kg to conform to current guidelines for retinal safety. The most recent recommendations from the American Academy of Ophthalmology advise a baseline screening examination within the first twelve months of treatment[115].

Leflunomide. Leflunomide is approved at doses of 10 mg and 20 mg once daily. We will use 20 mg daily as it is the most common effective dose.

4.3 Treatment Algorithm

- See Figure 2

Subjects randomized to the TNFi treatment arm will (based on randomization) receive either etanercept 50 mg SC weekly or adalimumab 40 mg SQ every other week (in addition to concomitant MTX and HCQ for subjects who entered the study on HCQ). Subjects will continue the same dose of their concomitant background MTX and HCQ, if applicable. If the subject's CDAI score is greater than 10 at 18 weeks, a good treatment response has not been achieved[116], then treatment will be switched to the other TNFi of interest for this trial. Subjects who initially started etanercept will be switched to adalimumab 40 mg SQ every other week. Subjects who initially started adalimumab will be switched to entanercept 50 mg SQ weekly. Subjects will remain on this new medication until the end of the study.

Subjects assigned to the triple therapy arm will begin SSZ 500 mg bid and HCQ 200 mg twice daily, not to exceed 6.5mg/kg HCQ (in addition to concomitant MTX). Subjects who enter the study on HCQ at a dose <200mg BID and are randomized to triple therapy will increase their dose to 200 mg BID HCQ, provided this new dose does not exceed 6.5mg/kg. At 6 weeks, SSZ will be increased to 1 g bid for all subjects. If the subject's CDAI score is greater than 10 at 18 weeks, a good treatment response[116] has not been achieved, and MTX will be switched to leflunomide 20 mg daily. The participant will remain on leflunomide (and concomitant SSZ + HCQ) for the remaining six weeks of the study.

4.4 Concomitant Medications

4.4.1 Corticosteriods

Patients who are not receiving corticosteroids and those receiving low dose steroids (≤ 10 mg/day of prednisone or equivalent) will be considered eligible for the study provided other entry criteria are met. Excessive dosages of steroids may suppress vascular FDG uptake independently of the randomized treatment. Prednisone (or equivalent) doses should not be altered by more than ±3.0 mg during the six months of the study. Two intra-articular injections of <40 mg triamcinolone (or equivalent) will be allowed during the study, but cannot be within one month of the second FDG PET/CT at the final visit. No oral corticosteroid "bursts" will be allowed unless there is reason that an intra-articular injection is impossible. If a burst is required, it can be no more than 10mg per day (prednisone equivalent) at maximum and no longer tha

n 14 days in duration before returning to +/- 3 mg of the original dose (or no use). Corticosteroid bursts cannot occur within one month of either of the FDG PET/CT scans.

4.4.2 Statins & PCSK9 Inhibitors

High-intensity statins and PCSK9 inhibitors have been shown to reduce arterial FDG uptake[52]. Therefore, patients who have been treated with a high-intensity statin or a PCSK9 inhibitor in the past 12 months or are currently on a high-intensity statin or a PCSK9 inhibitor are not eligible for the study. Patients taking a low-or moderate-intensity statin must be stable on their dose for six weeks prior to study entry and remain stable for the duration of the trial in order to eliminate a potential effect on arterial FDG uptake due to initiation or dose change of a low-dose statin. Patients likely to need a statin or PCSK9 inhibitor (e.g., insulin-dependent or uncontrolled diabetics and those with prior clinical CV events) are excluded from participation. Initiation of statins or PCSK9 inhibitors should be avoided if at all possible during the six month study period. If this is not deemed safe by the study site investigator and one of the two cardiologists working on the trial (Drs. Tawakol and Ridker), then the subject will be asked to come in for a follow-up FDG PET/CT before a statin or PCSK9 inhibitor is initiated. Subjects must have been on study treatment for at least 8 weeks in order for the FDG PET/CT to take place. If the subject has been on study treatment for less than 8 weeks, the subject will be dropped from the trial without completion of the follow-up PET scan. If a subject initiates a statin or PCSK9 inhibitor before a follow-up FDG PET/CT takes place, the subject will be dropped from the trial without follow-up imaging. This information will be included in the primary trial analysis.

4.5 Medication Monitoring

Two study investigators will take primary responsibility for Medication Monitoring (Drs. Liao and Giles). Both are board certified rheumatologists with experience using all of the study drugs. The ongoing integrity of the Medication Monitoring program will be assured by Drs. Liao and Giles. In addition, one of the two will always be on call to assist sites with medication related questions or concerns regarding side effects. A complete list of potential side effects can be found in the package inserts.

Per the American College of Rheumatology guidelines for the treatment of RA[4], we will obtain complete blood count, liver panel and basic metabolic panel on all subjects at six week intervals. Subjects will be instructed to bring in medication bottles to review medication adherence with site coordinators. Safety monitoring for each of the prescribed medications is well understood by practicing rheumatologists as these medications have been on the market for over a decade (TNFi's) or for several decades (SSZ and HCQ). Since this treatment study utilizes usual and approved medications for RA, standards of monitoring are well understood by the treating rheumatologists. If the study physician determines that a significant medication related toxicity has occurred, the drug can be temporarily withheld and restarted at the same or lower dose. If the toxicity recurs, the medication will be discontinued or the dose further lowered. If the medication is discontinued, the subject will be treated with the next drug listed in the treatment algorithm (Figure 2). See Section 4.5.1 for guidelines regarding adjustment of study medication doses due to lab abnormalities. In the case of the TNFi's, etanercept can be switched to adalimumab and vice versa. In the case of triple therapy, if one of the medications has to be discontinued, leflunomide 20 mg can be substituted before 18 weeks.

4.5.1 Abnormal Lab Values

All subjects recruited will have been on methotrexate and tolerated it. Thus, abnormalities in laboratory values outlined in the Table below will initially be assumed secondary to sulfasalazine in the triple therapy arm. As noted below in **Table 3,** two levels of laboratory abnormalities will be defined – caution and extreme values. Caution values will prompt re-testing in two weeks. If caution values persist, the total daily SSZ dose will be reduced by 500 mg and labs retested in two weeks; testing every two weeks will be continued and SSZ adjusted as needed until values normalize. If they do not normalize, then other factors including methotrexate will be assessed. Extreme values will prompt a temporary stop of sulfasalazine with re-testing at two weeks and be reported as an adverse event. If values remain in the extreme range on re-testing the subject will be withdrawn from the study. If extreme values improve to the caution range, then sulfasalazine will be resumed at a dose 500 mg lower than the previous dose and re-tested in two weeks.

Table 3: Caution and Extreme Values for Relevant Monitoring Laboratory Tests

	Est GFR (ml/min/1.7m2)	WBC (x1000n/uL)	Platelet (x1000n/uL)	Hct (%)	AST* (U/L)	ALT* (U/L)
Caution	30-40	3 - <3.5	50-75	<30-27%	>80	>112
Extreme	< 30	< 3	< 50	< 27%	>120	>168

* Local laboratory range. ULN, upper limit of normal.

4.5.2 Surgery or Infection

The following guidelines apply for subjects undergoing surgery during the course of the trial:

Emergency surgery	Temporary stop of study medications during surgery and during the post-operative period or until subject is clinically stable and off antibiotics
Planned surgery	Temporary stop of study medications for at least one week prior to surgery and during the post-operative period or until subject is clinically stable and off antibiotics.

The following guidelines apply for subjects experiencing infection during the course of the trial:

Acute infections	Hold study medications during the antibiotic course
Chronic infections	These subjects would be excluded, except if the chronic infection is diagnosed during the trial. If infection is diagnosed during the trial, then the risks and benefits of continuing trial treatment would be weighed on a case-by-case basis.

4.6 Study Procedures

4.6.1 General Overview

An overview of the schedule of clinical assessments and study procedures is outlined in Table I and detailed further in the MOOP. Briefly, a complete evaluation of the subject's RA history and of CV risk factors will be ascertained at the Screening and Baseline visits. Subsequent visits will be conducted every six weeks to assess safety and to determine whether a change in medication is indicated. Medication adherence will also be assessed using subject self-report and pill counts. Relevant study procedures and definitions are described below.

4.6.2 Joint Count

Assessment of the number of painful and swollen joints by a trained joint count assessor (an investigator or a trained research coordinator/nurse) is a standard element of all RA trials and of rheumatology clinical practice. Each of 44 joints (shoulders, elbows, wrists, MCPs, PIPs, hips, knees, ankles, MTPs) will be examined for tenderness (subject 'yes' or 'no') and swelling (metrologist 'yes' or 'no'). Although 44 joints will be examined, only 28 joints (shoulders, elbows, wrists, MCPs, PIPs, knee) are used to define the DAS-28 score (see below) and CDAI and will be used to calculate the EULAR 'good' response (see below). The information of all 44 joints is collected, however, because of interest in comparing clinically-defined level of joint disease activity vs level of FDG-PET/CT-defined joint disease activity.

4.6.3 RA Disease Activity

The 'DAS' (Disease Activity Measure) is a combined index to assess disease activity in RA[117] and is used routinely in clinical trials and clinical practice. It combines information from swollen joints, tender joints, the acute phase response (measured either by erythrocyte sedimentation rate or CRP), and general health into one continuous measure. A modification of this measure is the DAS-28 which includes only 28 of the total peripheral joints (shoulders, elbows, wrists, MCPs, PIPs and knees) and correlates well with assessments of more joints[118]. We will calculate the DAS-28-CRP at screening. During follow-up visits we will calculate disease activity using the Clinical Disease Activity Index (CDAI)[120] to evaluate treatment response. Good treatment response will be determined as CDAI ≤ 10, corresponding to low disease activity or remission[120].

4.6.4 CV Risk Factors

It will be confirmed during the Screening Visit that the subject has no self-reported, physician diagnosed prior clinical CV events. These include history of myocardial infarction, angina, stroke, heart failure, prior vascular procedure (coronary artery angioplasty or stenting, carotid endarterectomy, coronary artery bypass surgery, peripheral revascularization). In addition, patients with diabetes or fasting blood glucose >126mg/dl or non-fasting blood glucose >200mg/dl, and subjects who are currently or within the past 12 months have been treated with high-intensity statins or PCSK9 inhibitors are excluded. Once a patient is enrolled, the following conventional CV risk factors will also be assessed: 1) at each visit - blood pressure, serum glucose; 2) at the Baseline Visit – detailed smoking history, subject reported history of hyperlipidemia (if on a high-intensity statin for this, subject would have been excluded), physical activity, menopausal state, family history of heart disease, anthropomorphic measurements (weight, height, waist and hip circumference).

4.6.5 Laboratory Procedures

Screening labs (listed in Table 1) will be either performed at the study visit or abstracted from previous medical records if available and performed within the specified timeframe (see Section 3.5.2 Schedule of Study Procedures). Follow-up labs for medication safety monitoring will be performed at each visit, unless they are deferred in extreme circumstances (i.e. COVID-19 pandemic), in which case site PIs should use clinical judgement for the appropriate frequency of safety laboratory testing. However, this should be no less than every six months and no greater than every six weeks. Additional blood will be drawn for research (biomarker) purposes at the Baseline visit and at Visits 2, 3, 5 and 6; these will be collected in the fasting state. If samples collected for research are determined to be non-testable directly after their collection, the subject will be asked to come back in to have the research blood re-drawn as close to the original draw date as possible. Subjects must provide verbal consent for the re-draw.

Usual care safety laboratory measures at each visit are: complete blood count (CBC), basic metabolic panel (BMP), hepatic panel. Sites may choose to perform a comprehensive metabolic panel (CMP) if available, which includes both a BMP and LFTs.

Research laboratory measures. At the completion of the study, several analytes will be measured in the Harvard CTSA lab on batched subject samples from visits 2, 3, 5, and 6. These include rheumatoid factor (RF), anti-cyclic citrullinated peptide antibody (anti-CCP), high sensitivity CRP, lipids (total-, LDL-, HDL-cholesterol and triglycerides), and insulin and glucose. The latter two analytes will be used to assess level of insulin resistance. Measurement of these values in our research lab will ensure assay consistency across sites and subjects.

Research Biorepository. Whole blood will be sent directly from Clinical Sites to the BWH Biorepository. Sites that have processing capabilities, will processes some blood on site which will be sent frozen to the BWH Biorepository. Aliquots of sera and plasma will be processed at the Biorepository from the whole blood and stored for the above research laboratory measures and for potential Ancillary Studies. These samples will be frozen in 1 ml aliquots and

stored at -80C. Samples frozen at sites will also be stored by the Biorepository for future testing. All biospecimen procurement and processing will be overseen by the Biorepository at the BWH Division of Preventive Medicine (see Resources) and all aliquots samples will be stored, labeled and inventoried at this facility. A Laboratory Manual for the clinical sites will be prepared to ensure uniform and high quality preparation of the samples for mailing. The Biorepository will perform routine quality control on the freezers to ensure temperature control, back-up power, back-up cooled freezers in the event of power outage. The Biorepository will also be responsible for retrieving and shipping aliquots to other labs for specific analyte measurement (e.g., to the Harvard CTSA and to Crescendo Biosciences).

4.6.6 FDG PET/CT Scan

The FDG PET/CT scans will assess arterial FDG uptake in the ascending aorta and in the bilateral carotid arteries. Subjects will undergo FDG-PET/CT scans at baseline and between 24 and 36 weeks at their local facility. A detailed protocol prepared by the PIs of the Imaging Core (Drs. Tawakol and Fayad) is available as **Appendix B**. The procedure is summarized briefly here and is identical for the baseline and the follow-up scans. Subjects will fast for 10 hours the night before each of the two PET/CT scans and will abstain from carbohydrates, dairy, and fruit/candy at their last meal. The allowable foods after 5PM are limited to meat/fish/soy, eggs, green vegetables, cheese, water/coffee/tea (no milk, sugar). A serum glucose will be obtained at the imaging site prior to the scan and must be <150 mg/dL to proceed; if ≥150 mg/dL but <170 mg/dL, the serum glucose can be repeated in an hour to see if it is <150 mg/dL, if still ≥150 mg/dL the scan should be canceled & rescheduled as appropriate; if ≥170 at the initial reading, the patient should not be scanned and should be excluded from the study. This screening test may unmask undiagnosed diabetes which is an exclusion criterion for TARGET (A persistently elevated glucose indicative of newly diagnosed diabetes, coupled with the exclusion from undergoing the PET/CT scan, would make these subjects ineligible for the TARGET study).

If the subject's glucose is <150 mg/dL, he/she then receives the FDG injection and waits 90 minutes for the FDG to distribute through the body. The subject will sit quietly in the imaging waiting room (or other suitable nearby area) and limit muscle activity during this time. The subject will empty their bladder just before the scan is performed. At 90 minutes post-FDG injection, the CT attenuation correction scan, followed by the PET scan, for the chest is performed (Chest CT Scan and Chest PET Scan in the imaging protocol); this is followed by the CT attenuation correction and PET scans for the neck (Neck CT Scan and Neck PET Scan); this is followed by the CT attenuation correction and PET scans for the joints (Whole Body CT Scan and Whole Body PET Scan).

Sites will be required to perform a wet read after each FDG-PET/CT scan to look for any incidental findings of concern. This should be performed as soon as possible after the completion of the FDG-PET/CT. Sites will be required to report that this read took place and will need to report any findings of concern to the coordinating center via the EDC. Randomization will not take place until the wet read has been completed and it is confirmed that the subject can safely be randomized.

5. ADVERSE EVENTS

5.1 Definition of an Adverse Event

An adverse event (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

5.1 Serious Adverse Event

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

5.2 Relationship of an Adverse Event to Study Intervention

To assess the relationship of an event to study intervention, the following guidelines are used:

1. Related (Possible, Probable, Definite)
    a. The event is known to occur with the study intervention.
    b. There is a temporal relationship between the intervention and event onset.
    c. The event abates when the intervention is discontinued.
    d. The event reappears upon a re-challenge with the intervention.
2. Not Related (Unlikely, Not Related)
    a. There is no temporal relationship between the intervention and event onset.
    b. An alternate etiology has been established

5.4 Severity of an Adverse Event

The following scale will be used to grade adverse events:

1. Mild: no intervention required; no impact on activities of daily living (ADL)
2. Moderate: minimal, local, or non-invasive intervention indicated; moderate impact on ADL
3. Severe: significant symptoms requiring invasive intervention; subject seeks medical attention, needs major assistance with ADL.

5.5 Unanticipated Problems

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

5.6 Reporting of Adverse Events, Serious Adverse Events, and Unanticipated Problems

The DCC and Administrative Core will submit to the central IRB all adverse events. All adverse events that are unanticipated and related or possibly related to the research will be reported promptly to the BWH IRB. Sites will be responsible for reporting all events to the coordinating center via the EDC. Adverse events that are anticipated or unrelated will be reported to the BWH central IRB at the time of continuing review for all sites relying on the Partners HealthCare central IRB. Participants that sign a consent form will be considered enrolled until their participation is terminated or they withdraw consent. AEs will be reported on all those enrolled.

All Serious Adverse Events (SAEs) (regardless of expectedness, relatedness, or if they meet the definition for unanticipated problems) must be reported to the DSMB Safety Officer, and the NIAMS through KAI within 48 hours of the PI receiving notification of the event. The report should include a description of the event, as well as the Investigator's assessment of expectedness, relatedness and other information, as relevant. Any action taken by the investigative team should be provided in the report. The DSMB Safety Officer will be provided with this information but will provide an independent assessment on attribution and expectedness, as well as whether further action is recommended (e.g. collection of follow up information).

Anticipated adverse events are specified on the package inserts of all study drugs. Those adverse events that occur, which are not specified on the package inserts, will be considered unanticipated problems and will require reporting to the coordinating center and the IRB.

Sites using their local institutional IRBs will submit AE reports to the EDC, but must also report AEs to their own IRB according to institutional policies. Sites using the central IRB should adhere to the reporting timelines as follows: Reports for unanticipated adverse events are to be submitted within 5 working days/7 calendar days of the date the investigator first becomes aware of the problem. Initial reports for SAEs should be submitted within 24 hours of initial awareness of the event, with a complete report to follow within 7 calendar days of initial awareness.

All AEs (anticipated and unanticipated) will be collected, analyzed, and monitored by using the Adverse Event Form within the EDC, which will also be used to generate the AE tracking log. AEs and/or extreme 'value laboratory abnormalities identified in the protocol as critical to participant safety must be reported. All AEs experienced by the participant from the time of study enrollment through the end of study participation are to be reported. The site staff will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

Some incidental findings are likely to be identified in the PET/CT scans, or at other points throughout the study. Sites will be required to have an on-site radiology interpretation of the scan as soon as possible for safety purposes. The Site PI will be responsible for communicating any incidental findings to the participant's primary care physician. If the incidental finding is of an immediate nature (a medical emergency that requires immediate notification) such as suspected malignancy, notification to the primary care physician should occur within 24 hours of the availability of the on-site radiology report; urgent findings (abnormalities that require medical attention but not on an emergency basis) should be reported within 7 business days to the primary care physician. The Site PI will also be responsible for following up with this physician to determine whether any of the incidental findings will require withdrawal of the participant from the study. Incidental findings will only be recorded as AEs in the data collection system if they are considered to be related to study treatment.

The following delineates specific responsibilities of staff members:

- The **Site Research Coordinator** will complete the Adverse Event Form (and Unanticipated Problems on the corresponding form) in the EDC; assist the PI to notify the IRB and Safety Officer of all SAEs, and assist them to prepare SAE reports to IRB and/or the Safety Officer.

- The **Site Principal Investigator** will confirm that all AEs and UPs are correctly entered into the EDC by the coordinator; be available to answer any questions that the coordinators may have concerning AEs; assist the DCC and Administrative Core to notify the IRB, and/or the DSMB of all SAEs and AEs as appropriate. If the originating site is using their institutional IRB, the site will be responsible for submitting the adverse event report according to institutional policies, with assistance from the DCC and Administrative Core as needed.

- The **DCC and Administrative Core Staff** will confirm that the AEs are correctly entered into the EDC. They will confirm that the adverse events are consistent with the source documents and are reported to the appropriate regulatory bodies, as required. If the originating site is using the central IRB, the DCC and Administrative Core will submit the adverse event report to the central IRB. Additionally, the DSMB will receive safety tables every 6 months during the study period that include AE and SAE reports.

6. TRAINING AND MONITORING OF SITES

6.1 Training of Site Investigators and Staff for the Study Protocol

Site training will be done by Webinar. All sites will be required to complete a set of training modules prior to recruitment, on the following topics: Introduction to the Study, Study Protocol, Study Recruitment and Consent, Study Drugs, the EDC, Imaging Protocol, Joint Assessments, Labs, Monitoring and Reporting, and Abnormal Situations. All of these modules will be delivered as live webinars. Each webinar will be recorded and available for download from the trial website. Study coordinators must attend or view all of the modules. Site PIs and co-investigators will need to view several modules, including: Introduction to the Study, Study Protocol, Monitoring and Reporting, and Abnormal Situations. At the webinars, key components of the trial design and implementation will be presented, and site staff will have the opportunity to ask questions. Topics that will be covered include protocol details, recruitment, inclusion/exclusion criteria, EDC training and joint count assessment. Sites will also receive additional individualized support while enrolling their first participants in the form of frequent phone calls from Central staff as well as the opportunity to complete the first visits while central staff are on the phone to answer any questions. DCC staff will also be available for personal site training on the EDC as needed and to answer questions as they arise.

The investigators and all staff involved in the study will have completed their required Collaborative IRB Training Initiative (CITI). Each study staff member will be trained in the protocol and specific procedures for each study visit by the study investigators. New study staff members will be trained on the protocol and, if necessary, spend a visit shadowing another trained staff member before carrying out protocol tasks on their own. Prior to conducting subject visits, investigators will be asked to sign off that the site staff members have been appropriately trained in the study protocol.

Training and delegation of responsibility will be documented in the Delegation of Responsibility Log and tracked via the trial website (where the training modules will be posted).

6.2 Training for Electronic Data Capture

One of the key training modules will focus on use of the EDC. Topics covered will include:

- Appropriate use of credentials to log-in
- EDC security
- Navigating the EDC
- Recording information on the eCRFs
- Screening
- Enrollment and Baseline
- Randomization
- Concomitant Medication
- Follow-up Visits
- Source documentation
- Submitting adverse event reports
- Revising data and audit trails

Additional one-on-one training will be provided to sites on an as-needed basis, as well as guidance for each site to get registered on the EDC.

The EDC also includes an online EDC Manual, which provides information on EDC functions as well as additional documentation for the study forms. These will be available to study staff when logged into the EDC system.

6.3 Training for Joint Count Assessment

Only sites that already have trained and experienced joint count assessors will be invited to participate. In the event that the joint count assessor has not been trained or a refresher is needed, a video of a standardized joint count assessment for swollen and tender joints will be made available to the sites.

6.4 Imaging Core Training

To ensure consistency in scan acquisitions across all imaging centers, a primary technologist will be appropriately trained by Imaging Core (in the imaging protocol also called the Icahn School of Medicine-Massachusetts General Hospital ISMMS-MGH Imaging core (IMIC)) on all relevant aspects of this study prior to scanning study subjects. The site Imaging Centers are also encouraged to identify a back-up technologist who will undergo the same training. The Imaging Core will require PET/CT Technologists to participate in training via webinar prior to the initiation of the study. All study coordinators will be required to complete a training module about the imaging that will provide important information they will need to convey to the patient about scan day. Additional Investigators Site personnel will also be invited and are encouraged to attend the training session.

Training will be held via online webinar. The Imaging Core will provide login information prior to all training sessions. Additionally, each Site Imaging Center will be required to submit image sets of standardized PET/CT phantoms prior to approval for imaging. Initial phantom data will undergo a preliminary quality control (QC) review by the imaging core team to ensure that images transferred are in proper format, that both PET and CT series are present, and the images appear to be of acceptable quality. This will ensure correct application of training principles.

A protocol for the FDG PET/CT is provided in **Appendix B.**

6.5 Monitoring of Sites

Monitoring will occur throughout the study period. Internal reports will be generated regularly to identify missed visits, missing data, and other data clarifications. Additionally, automatic queries will be programmed into the EDC to identify

missing data at the time of visits entry and out of range values. Reports examining consistency of responses of time will also be generated and clarification requested as necessary. Virtual monitoring will also occur throughout the study. Core staff will periodically request that a site provide additional documentation and regulatory files. This documentation will be mailed, or faxed by the site and reviewed at the Coordinating Center.

The DCC will be able to track a site's progress via the EDC. Should a site consistently deviate from the protocol or not perform to a sufficient level, the DCC will recommend intervention by the Administrative Core. The Administrative Core will investigate the source of the deviations, and will provide additional training or coaching as needed, as well as decide to drop an enrollment site if necessary. While existing enrollment sites may be dropped for substandard performance, new enrollment sites may be added during the course of the study. Many of the investigator trainings will be recorded, and the Administrative Core will facilitate support for one-on-one site trainings as needed.

6.5.1 Clinical Sites

Use of the EDC will enable frequent monitoring of study sites to ensure protocol adherence. The DCC will have immediate access to all data that is entered on the EDC, and will be alerted to any issues, including the ones listed below:

- Non-compliance with protocol
- Missing data
- Out-of-range data
- Out-of-window visits
- Data clarification
- Consistent and logical dates over time
- All fields of a "completed form" actually completed or reason for no data noted
- All required forms completed or reason for no data noted
- Data consistent across forms and visits

The DCC will provide regular protocol adherence and data monitoring reports to the Administrative Core, and the Administrative Core will pursue corrective action from sites as needed. As well, the EDC will be developed in such a way that sites will be alerted to correct out of range values or missing data as they are entering it, thereby reducing the occurrence of data and protocol violations.

6.5.2 Imaging Sites

In addition to the initial quality check of phantom images used to train and certify imaging sites, the imaging core has a well developed and successful protocol for monitoring image quality. Details are provided in the imaging protocol **(Appendix B).** Briefly, upon receipt of PET/CT images to the Imaging Core within 1 day of image acquisition (via FTP transfer), a Clinical Research Associate (CRA) confirms image set completeness and proper labeling/anonymizaton. Quality control (QC) is performed to verify that the image acquisition guidelines were followed. If the CRA identifies an artifact that could impair the ability to interpret images, the Site Imaging Center is notified of actions that need to be taken. The Site is required to resolve the query and/or send the

missing/discrepant information to the Imaging Core within two business days. Once resolved, an Acceptance Notification is issued. Any deviation from the protocol, or change in parameters, must be pre-approved by the Imaging Core in advance and noted on the Image Record Forms.

The FDG PET/CT and image reader case report forms (CRF) will be used to assess adherence to the imaging protocol. These data include: a) tracer circulation time; b) intra-subject difference in circulation time (between scans); c) fasting blood glucose; d) injected minus residual FDG activity; e) scanner used; f) arm position; g) reconstruction method; h) time per bed position; i) acquisition mode; j) slice thickness.

Image Quality will be graded as excellent, marginal, and unacceptable. Data analysis will be performed first on data derived from excellent quality scan only, then excellent and marginal quality scans combined.

7. STUDY OBJECTIVES AND STATISTICAL ANALYSIS PLAN

7.1 Primary and Secondary Objectives

The primary objective is to compare the effects on vascular inflammation of TNFi + MTX ± HCQ versus triple therapy in subjects with RA who are inadequate responders to MTX using FDG-PET/CT as a tool for detecting vascular (arterial) inflammation.

The secondary objective is to compare the effects on vascular inflammation of achieving low disease activity or remission (LDAR) versus moderate-high disease activity (MHDA).

The exploratory objective of the TARGET Bioethics Ancillary study is to gather data on researchers' attitudes and beliefs regarding incidental findings and the prevalence of incidental findings from whole body FDG PET/CT in RA.

The exploratory objective of *the Neuroimaging* Measures Ancillary Study is to investigate associations between stress-associated neurobiological activity and articular and arterial treatment response in RA.

7.2 Study Hypothesis

The primary aim of the Treatments Against RA and Effect on FDG PET/CT (TARGET) trial is to test whether treatment with a TNF inhibitor (TNFi) in addition to methotrexate (MTX) with or without concomitant HCQ (only for subjects entering the study on concomitant HCQ) among RA subjects who are inadequate responders to MTX reduces cardiovascular inflammation compared to triple therapy (MTX+SSZ+HCQ) as measured by the mean of the maximum target-to-background ratio (TBR) of FDG uptake at the 6 month follow-up for the most diseased segment (MDS) identified at baseline.

The primary hypothesis is that TNFi +MTX + HCQ (only for subjects entering the study on concomitant HCQ) will reduce vascular inflammation to a greater extent than triple therapy when measured at 6 months.

Additional planned analyses in the second aim test whether the follow-up value in cardiovascular inflammation is correlated with measures of disease progression. The markers of disease progression include: 1) disease activity as measured by the DAS28-CRP score, with achievement of low disease activity or remission considered to be a DAS28-CRP score of less than 3.2 at the 6-month follow-up (all participants enter the trial with a DAS28 above 3.2); 2) a commercially available MBDA (Vectra-DA) which will be measured at baseline and 6 months; and 3) joint inflammation defined as the average standardized uptake value (by FDG PET/CT) in the same joints used in the calculation of the DAS-28, and measured at baseline and 6 months using the images collected for the primary endpoint as described in the Imaging Section.

The secondary hypotheses are as follows:

- We hypothesize that subjects in DAS-28 defined LDAR at 6 months will have less vascular inflammation than those with persistent MHDA.
- We hypothesize that subjects in Vectra DA-defined LDAR at 6 months will have less vascular inflammation than those with persistent MHDA.
- We hypothesize that subjects in articular FDG defined LDAR at 6 months will have less vascular inflammation than those with persistent MHDA. We will also compare the correlations of the articular FDG uptake and DAS-28 with vascular inflammation to determine if they are independent.
- We hypothesize that the exploratory objective of the TARGET Bioethics Ancillary study will help develop clear policies and procedures for reporting and managing incidental findings in imaging research, refine the informed consent process and standardize reporting, evaluation, and follow up of incidental imaging findings.
- We hypothesize that the higher levels of stress-associated neurobological activity, measured objectively as rAmygA, associate with a blunted response to RA immunomodulatory treatment in the joints and vasculature as assessed with measures of articular response (i.e. patient self-assessment and change in joint counts) and objectively with change in articular and arterial FDG uptake.
- We hypothesize that the link between stress and blunted response will be reflected in enhanced hematopoietic tissue activity in the bone marrow, and in circulating inflammatory biomarkers.

7.3 Study Design

TARGET is a randomized, open-label, multi-center trial. Eligible participants will be randomly allocated, 1:1, to TNFi + MTX + HCQ (only for subjects entering the study on concomitant HCQ) or triple therapy. Baseline imaging will be done at the time of randomization and subjects will be followed for 6 months, at which time the follow-up imaging will be done.

TARGET Investigators will be surveyed via a REDCap link regarding their attitudes and beliefs surrounding the return of incidental findings from whole body PET/CT scans..

7.4 Number of Subjects

We plan to randomize 200 participants to achieve an effective sample size of 170 people with complete follow-up and measurements of both the baseline and 6-month MDS TBRmax. We estimate over 90% power to detect a difference of 0.15 in 6-month MDS meanmax TBR between the two arms, even allowing for a 10% dropout rate. A between group difference of 0.15 also corresponds to the expected observed effect if the between group difference were 0.17 and there was a 10% crossover rate.

Sample size and power were calculated using several alternative scenarios for dropout rates and cross over. All cross over was assumed to be from the triple therapy arm into the TNFi arm and these participants would be included in the main intention-to-treat analysis set under their assigned treatment group. Drop outs consist of participants for whom imaging results were unavailable at the baseline or 6-month timepoint. These participants would be unable to contribute to the analysis and would directly reduce the sample size available. Previous data in the RA population found a baseline MDS meanmax TBR of 2.51 (SD of 0.33) and a 0.46 reduction after 8 weeks of a TNFi (correlation of 0.5 between baseline and 8 weeks).

The method that we intend to use for our primary analysis is an ANCOVA model estimating the final index vessel MDS meanmax TBR as a function of baseline index vessel MDS meanmax TBR and treatment group. The resulting treatment group coefficient will then estimate the change in MDS meanmax TBR associated with treatment assignment after adjusting for baseline differences.

Power for the ANCOVA analysis was calculated using the sampsi function in Stata. This function uses the method laid out

by Frison and Pocock in Statistics in Medicine in 1992. We performed the power calculations using a correlation between the baseline and final MDS TBR meanmax of conservative correlation estimates of 0.4 and 0.3 and have included the results in the Tables 4A and 4B below.

TABLE 4a: Power Calculation for Aim 1 for Correlation of 0.4 (ANCOVA adjusting for baseline)

	Dropout Rate		
Between group difference	0% (N=200)	5% (N=190)	10% (N=180)
0.17	98%	97%	96%
0.16 (5% crossover into TNFi)	96%	95%	94%
0.15 (10% crossover into TNFi)	94%	93%	91%

TABLE 4b: Power Calculation for Aim 1 for Correlation of 0.3 (ANCOVA adjusting for baseline)

	Dropout Rate		
Between group difference	0% (N=200)	5% (N=190)	10% (N=180)
0.17	97%	96%	95%
0.16 (5% crossover into TNFi)	95%	94%	93%
0.15 (10% crossover into TNFi)	92%	91%	89%

Power for the secondary aims

The secondary aim compares 6-month MDS meanmax TBR by LDAR achievement. The primary analysis will use ANCOVA to adjust for baseline MDS meanmax TBR as well as other baseline characteristics related to LDAR achievement (age, gender, disease duration, smoking status, serologic status) and treatment assignment. Assuming 30% reaching LDAR in both groups[111-113], and the parameters of MDS meanmax TBR from Aim 1, we use a t-test of 6-month MDS meanmax TBR as a conservative power estimate. The proposed trial will have 90% power to detect an absolute difference between those reaching LDAR and those not of 0.18 in MDS meanmax TBR with a dropout rate of up to 10%; this would be clinically significant based on the aforementioned statin study[52].

TABLE 5: POWER CALCULATION FOR AIM 2	
	Dropout Rate

Between group difference	0% (N=200)	5% (N=190)	10% (N=180)
0.18	94%	93%	92%
0.17	91%	90%	88%
0.16	88%	86%	84%

7.5 Analysis Sets

The primary analysis for efficacy will be defined as all randomized subjects with complete imaging results at baseline and 6 months, according to their randomized treatment assignment. Safety analyses will be done using all subjects entering the study, according to the treatment received. Per-protocol analyses will also be done for participants who remained on their randomized treatment assignment for the duration of follow-up.

7.6 Safety Analysis

All participating subjects will be included in safety analyses, regardless of their completion of the imaging studies. The two arms of the trial both use standard of care treatments, so serious unanticipated adverse events are unlikely. Reports of adverse events by randomized treatment assignment and actual treatment will be generated for the DSMB at regular intervals of approximately 6 months. We will also present frequency analyses for withdrawals and compliance with protocol.

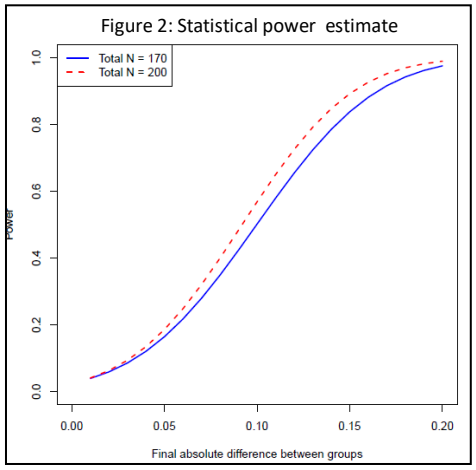
7.7 Analysis Methods

Aim 1:

The main analysis for Aim 1 will use ANCOVA to compare the 6-month arterial MDS meanmax TBR between assigned treatment groups, adjusting for baseline values and statin initiation, with a null hypothesis of no association between treatment assignment and 6-month MDS meanmax TBR. A p-value threshold of 0.05 for a two-sided test will be used to determine statistical significance. Additional analyses will explore secondary imaging measures including meanmax overall TBR. We will also repeat the main analysis in the subgroup per-protocol compliance. Other important subgroups will also be analyzed within assigned treatment groups using an interaction term, including achievement of LDAR, serologic status, known CV risk factors, and excluding statin initiators.

The primary analysis will be based on assigned treatment group, only including participants with imaging data at baseline and 6-month follow-up. A sensitivity analysis for the impact of any missing data will also be done using the same assigned treatment categories, but using all randomized participants with **[**multiple imputations using a Markov chain Monte Carlo technique[119]. We anticipate few missing data because: 1) adequate staff (2 Research Assistants) in the Administrative Core will closely monitor weekly data entry; 2) 24-week trial allows for detection of significant differences in vascular FDG PET/CT but will reduce drop-out that may occur in a longer trial; 3) recruitment of experienced sites with site coordinators; 4) providing transportation reimbursement to subjects whenever necessary; and 5) providing reminder mailing cards to sites to send to subjects. 2 secondary analyses were added based on reviewers' comments: excluding subjects who escalated dosing; and, in an exploratory secondary analysis, we will compare the 6-month MDS meanmax TBR between adalimumab and etanercept users.

Sample Size: Power to detect alternate absolute differences in meanmax TBR is presented in **Figure 2** for a study with 100 each arm (200 total randomized target). Previous data in RA baseline MDS meanmaxTBR of 2.51 (SD of 0.33) and a 0.46 after 8 weeks of a TNFi[62]. Using a t-test to compare MDS in the two arms at 6-months generates a conservative power With an effective sample size of 170 (200-30; 15% drop-out or the proposed trial has a 90% power to detect an absolute 0.17 between the 2 arms. This difference corresponds to the observed in the prior study by Maki-Petaja for TNFi's[62]. It clinically important difference, on the same order for what between a low-dose statin and a high-dose statin[52], a contrast clinical significance[53]. **[**Power will be sufficient for secondary endpoints. The pre-specified secondary analysis focusing on approximately 75% of subjects remain on the original drug dosages will include an estimated 64 subjects per arm and will have 90% power to detect a difference of 0.19 in MDS meanmax TBR. Since these are pre-specified secondary endpoints, p-values will not be adjusted.**]**



6 month MDS participants in pts found a reduction meanmax TBR estimate. cross-over), difference of effect would be a was observed with known

Aim 2a:

This aim compares 6-month MDS meanmax TBR by LDAR achievement. The primary analysis will use ANCOVA to adjust for baseline MDS meanmax TBR as well as other baseline characteristics related to LDAR achievement (age, gender, disease duration, smoking status, serologic status) and treatment assignment. Using a conservative estimate of 30% reaching LDAR in both groups[111-113], and an effective sample size of 170, we ran a t-test of 6-month MDS meanmax TBR as a conservative power estimate. The proposed trial will have 90% power to detect an absolute difference between those reaching LDAR and those not of 0.19 in MDS meanmax TBR; this difference would be clinically significant based on the aforementioned statin study[52].

Aims 2b and 2c: These exploratory aims will examine the relationship between the Vectra-DA and articular FDG uptake (respectively), and 6-month change in arterial MDS meanmax TBR. The analytic strategies will be very similar to Aim 2a. Relationships between Vectra-DA disease activity categories and articular FDG PET/CT disease activity and 6-month change in MDS meanmax TBR will be analyzed similar to Aim 2a. The statistical power for Aims 2b and 2c will be very similar to Aim 2a. Exploratory analyses will examine: 1) alternative thresholds for disease activity using the Vectra-DA and articular FDG PET/CT; 2) using the Vectra-DA and articular FDG PET/CT as continuous measures; and 3) including the Vectra-DA and articular FDG PET/CT continuous measures as covariates in the ANCOVA conducted for Aim 2a.

7.8 Data Management

7.8.1 Clinical Data

Most study forms will be completed using an EDC system that is managed by the DCC. The EDC system is a web-based software application where all study data from sites will be recorded, managed, and stored. Data will be collected by site study staff and, where possible, entered directly into the electronic case report forms (eCRFs) housed on the EDC. When data are first recorded through the EDC, the EDC record shall serve as the source document. The eCRFs will replace traditional paper CRFs, and should be used to capture data for all enrolled subjects. This model has been used in other trials and has resulted in low rates of data errors and protocol violations, and rapid resolution of problems. The EDC will be hosted and maintained by StudyTRAX (ScienceTRAX, Macon, GA). In addition to security procedures including encryption, prevention of data corruption and multiple secure backups, the EDC will be FDA regulation 21 CFR part 11 and HIPAA compliant.

Once recorded in the EDC, the data is immediately accessible by the DCC for review, verification, data queries, and to be locked when appropriate. Review of the data will allow the DCC to monitor protocol adherence and ensure clean data, as well as addressing problems in this regard as they arise. The EDC will also be instrumental in generating reports, including recruitment progress, status of follow-up visits, and adverse event tracking. Automatic and manual data checks will be performed routinely.

Whenever original observations and data are first collected and entered directly into the EDC system, the electronic record is the source document. The EDC system will serve as source documentation for most forms in this protocol. However, if a visit is performed using paper forms and those papers are used to later enter data into the EDC, the paper forms will be the source documentation and must be kept for records. We will not permit the substitution of the EDC with paper forms. We will allow sites to keep notes and then enter information within 72 hours into the EDC.

Prior to any subject enrollment, each site will receive training and guidance from the DCC to be active on the EDC. As a web-based application, the EDC system will be accessible through any computer or device with internet access and a non-mobile web browser. No downloads or installations are necessary to access the EDC. Each member of the study staff who interacts with the EDC to collect or verify data will be issued a unique username and password, and staff will log-in using those credentials any time data is entered or modified. In this way, the EDC will track changes and create an audit trail for each piece of data. The Delegation of Responsibility Log will be maintained through the EDC, and it will be used to designate roles and access for each study staff member.

All study records will be retained for seven years (or longer if required by local IRBs).

7.8.2 Imaging Data

Imaging data will be managed by the Imaging Core. Imaging data will be transferred to the Imaging Core over a secure FTP server. All images will be assigned a unique ID number and are anonymized at the Site Imaging Centers. Details are provided in the Imaging Protocol (**Appendix B**).

7.9 Handling of Missing Data

The primary analysis will be based on assigned treatment group and will only include participants with imaging data at baseline and the 6-month follow-up. A sensitivity analysis for the impact of any missing data will also be done using the same assigned treatment categories, but using all randomized participants with all missing baseline values set to the trial average and all missing change values set to zero (or no change). We anticipate few missing data because: 1) adequate staff (2 Research Assistants) in the Administrative Core will closely monitor weekly data entry ; 2) 24-week trial allows for detection of significant differences in vascular FDG PET/CT but will reduce drop-out that may occur in a longer trial; 3) recruitment of experienced sites with site coordinators; 4) providing transportation reimbursement to subjects whenever necessary; and 5) providing reminder mailing cards to sites to send to subjects.

7.10 Bioethics Ancillary

We will survey the TARGET investigators to determine researchers' attitudes and beliefs regarding their ethical obligation to return and manage incidental research findings from whole body PET/CT imaging studies and determine the prevalence of incidental research findings on whole body FDG PET/CT imaging in rheumatoid arthritis (RA) and detection rate of previously unknown malignancies. There is a lack of information available on researchers' attitudes

and beliefs regarding incidental research findings and the prevalence of incidental research findings from state of the art imaging studies, such as whole body FDG PET/CT in those without known malignancy, and to our knowledge, no such information available in rheumatology. The Treatments Against RA and Effect on FDG PET/CT (TARGET) study provides an unprecedented and unique opportunity to examine the ethics around incidental findings from whole body FDG PET/CT in RA patients without known malignancy. Gathering data on researchers' attitudes and beliefs regarding incidental findings and the prevalence of incidental findings from state of the art imaging studies will help develop clear policies and procedures for reporting and managing incidental findings in imaging research, refine the informed consent process and standardize reporting, evaluation, and follow up of incidental imaging findings.

7.11 Neuroimaging Measures Ancillary Study

PET/CT **Brain image analysis,** is performed while blinded to subject data and temporal identifiers, using a dedicated workstation that enables multi-modal standard image fusion (Leonardo–TrueD, Siemens Solutions). The amygdala is localized on the CT images using anatomic landmarks, as described previously[112]18F-FDG uptake in amygdala was determined by placing a circular region of interest (ROI) in the defined structure bilaterally, and mean and maximum tracer accumulation were recorded as standardized uptake value (SUV) for each ROI. Amygdalar activity is corrected for brain background activity, using cerebral (temporal lobe). The primary analysis for the study was the average of the mean right and left amygdalar SUVs divided by the cerebral background.

PET/CT **Bone marrow and splenic activities**, are measured according to previously validated methods[127]. Bone marrow ^{18}F-FDG uptake was determined by placing ROIs in axial sections of individual vertebrae from T1 to L5. The maximum SUV was recorded for each vertebra, and bone marrow activity was calculated as the average of the registered SUVmax of all measured vertebrae. Splenic ^{18}F-FDG uptake was determined by placing a ROI in each of axial, sagittal, and coronal sections. The SUVmax was recorded for each section, and splenic activity was calculated as the average of the three registered SUVmax values.

8. REGULATORY CONSIDERATIONS

8.1 Informed Consent

Site investigators are responsible for obtaining informed consent before any subject may participate in the TARGET trial. Informed consent requires adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. No protocol-specific screening procedures or any study medications will be prescribed prior to obtaining informed consent. Acquisition of informed consent will be documented in the subject's medical records and the informed consent form should be signed and personally dated by the subject as well as by the person who conducted the informed consent discussion. The signed consent form will be retained according to institutional policy. A copy of the signed consent form will be provided to the subject. Ancillary studies that require study procedures additional to the parent TARGET Trial will require separate consent forms. The subject may withdraw consent to participate in the study at any time. For the bioethics survey, the completion of the survey will serve as the consent for the TARGET investigators who participate.

8.2 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be appointed by the NIH to monitor and evaluate safety and scientific issues related to the study. We anticipate that its membership will include: a biostatistician; a cardiologist with trial experience; 1-2 rheumatologists with trial experience; and a bioethicist. Persons outside of CUMC and BWH and who have no conflict of interest with the TARGET Study will be chosen to assure independence. The DSMB will meet before the study starts and every 6 months thereafter; additional ad hoc sessions can be convened in the event of more urgent issues. The DSMB will compile and approve a written charter. The NIH Project Officer will attend the DSMB meetings and will receive copies of all reports. The biostatistician will be the DSMB Executive Secretary and one member,

designated by NIAMS, will serve as DSMB chair. Topics to be discussed at DSMB meetings are recruitment, adverse events, and consideration of protocol modifications to assure safety. DSMB members will be blinded to treatment assignment when monitoring safety data, unless an event necessitating un-blinding occurs. These blinded (and if necessary unblinded) reports will be provided to them quarterly by the DCC.

In addition to participant safety, the DSMB will also be charged with data monitoring. For this, the DSMB must: ensure data integrity and confidentiality, assist NIAMS by commenting on any problems with study conduct or enrollment, sample size, statistics, and/or data collection; and review and evaluate requests for protocol modifications after the trial begins.

8.3 Institutional Review Board

The study will be conducted at each site under the monitoring of a local or central Institutional Review Board (IRB) (depending on the individual sites capabilities). Partners HealthCare IRB at Brigham and Women's Hospital has been selected as the central IRB and will oversee IRB responsibilities. This protocol has been approved by them.

Each Site PI will be responsible for submitting the clinical trial protocol, the Informed Consent form, any advertisements, and all other relevant study related documents to their local IRB for approval. For sites using the trial's central IRB, these documents will be submitted through the Administrative Core. Documentation of each site's IRB approval must be provided to the Administrative Core before study enrollment can commence. All unanticipated problems, as well as protocol deviations and violations~~,~~ that occur during the conduct of the trial will be reported by the Site PIs to their respective IRBs and to the TARGET Administrative Core. A full list of all adverse events (most of them will not be unanticipated) will be submitted regularly to the DSMB and the responsible IRBs. The IRB must approve all protocol changes prior to implementation unless emergency action is clinically indicated. Site PIs will be responsible for preparing annual reports to the IRB (or more frequently if required locally) and a final report upon study completion. Support for the Sites for these IRB functions will be provided by the Executive Committee.

8.4 Pre-Study Documentation Requirements

Before a site not relying on the central IRB can begin enrolling, the Administrative Core must receive the following documents:

- Copy of stamped IRB approved consent form
- Copy of the IRB approval letter for the protocol and consent form.
- CV of the PI and co-investigators
- Signed and dated protocol signature page

Before a site that is relying on the central IRB can begin enrollment approval must be granted from the BWH central IRB for the addition of the site.

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APPENDIX B:

Imaging Protocol

Imaging Protocol

Treatments Against RA and Effect on FDG PET-CT: The TARGET Trial

Imaging Core Principal Investigators:

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Version Number: 6.0

4 December 2017

ATTESTATION PAGE

Protocol v6.0, 4 December 2017

The below signature of the physician overseeing the imaging at the TARGET Trial Imaging Site attests that this imaging protocol has been reviewed and that the imaging site is in agreement with the TARGET Trial Radiation Safety Monitoring Plan (see Appendix D).

Imaging Site: _____

Imaging for TARGET Clinical Site: _____

Physician Overseeing TARGET Trial Imaging or Radiation Safety Officer (print name):

_____

Email: _____Phone #: _____

I attest that I have reviewed the TARGET Trial Imaging Protocol and agree to the TARGET Trial Radiation Safety Monitoring Plan. I understand that a safety read for incidental findings will determine TARGET eligibility and that I will provide a safety read for incidental findings within 72 hours to the clinical site involved in the TARGET trial.

Signature: _____

Date: _____

Table of Contents

ISMMS-MGH Imaging Core Charter

1. Imaging protocol

ISMMS-MGH Imaging core (ICL) has developed the following acquisition guidelines for standardization of the study imaging components across imaging centers participating in the TARGET trial. Provided here are image acquisition guidelines for 18-FDG-PET/CT imaging of vascular inflammation. The protocol provides a guide for participating centers and acknowledges that variations in the technical abilities of the scanners used may introduce minor variations in the settings used for this study.

Regularly scheduled imaging for this study should be acquired in strict adherence to these guidelines. Please ensure the blinding of all confidential subject/site information on all images. Only combination PET/CT scanners should be used to acquire data for this study. Please notify ICL immediately of any changes (including upgrades) to the PET/CT scanner hardware or software.

The table below shows the schedule for the imaging timepoints for evaluating vascular inflammation:

		Baseline Visit	**Followup Visit**
Modalities		^{18}FDG-PET/CT	^{18}FDG-PET/CT
Anatomy of interest	Chest (aorta)	CT Scan & PET Scan	CT Scan & PET Scan
	Neck (carotids)	CT Scan & PET Scan	CT Scan & PET Scan
	Peripheral joints	CT Scan & PET Scan	CT Scan & PET Scan

Protocol Overview

Scan	Coverage	Positioning	Minutes per scan
Scan 1 (Chest)	Shoulders to diaphragm	Arms up	Approx 12.5
Scan 2 (Neck)	Top of eyes to sternal notch	Arms down	Approx 2.5
Scan 3 (Joints)	Above shoulders to toes	Arms down	Approx 28
	Time between scans		Approx 7
	Total Time		Approx 60

1.0 IMAGE ACQUISITION GUIDELINES, PRE-IMAGING PREPARATION, 18FDG-PET/CT

- **All subjects need to adhere to an induction phase low-carbohydrate diet (see Attachment A: Dietary Instructions for Subjects), especially for their dinner meal preceding the FDG-PET scan.**
  - **The clinical site will provide the subject with this information.**
- **Subjects should be encouraged to drink plenty of water while fasting.**

	Instructions
Morning Imaging	Subjects being imaged in the morning must have fasted for at **least 10 hours the previous night.**
Afternoon Imaging	Subjects being imaged in the **afternoon must have fasted for at least 10 hours** the previous night but may have a breakfast from the recommended menu and then **fast for at least 4 hours** prior to the ^{18}FDG injection.
~5 minutes prior to IV injection of ^{18}FDG	A blood glucose (BG) measurement (mg/dl) will be taken*: • if BG<150: proceed with imaging • if BG>150 but ≤ 170: reschedule or may repeat BG within 1 hr to see if it comes down to <150 i.if BG is still >150, patient should not be scanned and should be excluded from the study – site coordinator should contact TARGET Central • if BG>170: patient should not be scanned and should be excluded from the study – site coordinator should contact TARGET Central

*Glucose measurements should be made using a CLIA approved, CLIA cleared, or equivalent glucose measurement technique.

- **Synchronize clocks**
  - **Ensure clocks used to record times for the study procedures on the imaging** day are synchronized with the scanner and dose calibrator clocks.

- **Obtain Subject height and weight**
  - Record subject height (without shoes) at first visit
  - Weigh subject (without shoes, jackets or sweaters) and record weight at each visit.

- All pre-imaging steps should be followed:
  1. Obtain IV access

  2. Check fasting blood glucose (mg/dl)
     - if glucose<150: proceed with imaging
     - if glucose 150-170: reschedule or may repeat BG within 1 hr to see if it comes down to <150
       i. if glucose still >150: patient should not be scanned and should be excluded from the study
     - if BG>170: patient should not be scanned and should be excluded from the study – site coordinator should contact TARGET Central

  3. Draw 10 mCi of ^{18}FDG and assay with a dose calibrator.
  a. Record on the Image Record Form (IRF):
     i. Assay time to the nearest minute
     ii. Initial ^{18}FDG dose (in mCi) to the nearest tenth.

  4. Inject the ^{18}FDG through the subject's IV line. Record this time to the nearest minute.

  5. Flush the syringe and line with approx 20cc of normal saline.

  6. Repeat assay of syringe and measure residual activity within syringe.

  7. Record ^{18}FDG injected dose (initial-residual activity) to the nearest tenth of mCi.

- **Target circulation time is 90 minutes**
  - Shortly before bringing the subject to the scanner, have the subject use the restroom to empty the bladder.
  - Subject should be placed in the PET/CT scanner **approximately 10 minutes** before imaging should commence. Ask the subject to remain reclining or supine in a quiet, room temperature area.
  - The subject may watch television but should limit muscle activity (advise against reading, conversing, chewing, etc.).
  - Place warm blanket on subject to reduce brown fat activation.
  - **Scanning should commence 90 minutes after injection**

Important:

For subsequent PET/CT visits, make sure to repeat the following parameters:

- Same FDG dose (± 5%)

- Same FDG circulation time (± 10 minutes)
- Same time of imaging (time of day ± 60 minutes)
- **For the 2nd Scan (Visit 6), CT Parameters will depend on BMI at the initial imaging visit -** Acquisition will not change based on a subject gaining or losing a few pounds.

1.1 PET/CT Chest

Chest – CT Scan

Subject Orientation:	• **Arms UP**, Supine position. • Use the sagittal laser and the subject's nipples as a landmark to ensure that there is no rotation of the chest to either side. • Velcro straps and/or tape may also be used to maintain subject positioning. • Mid-breath breath hold or quiet breathing

Scan Locations/Coverage:	• Entire chest
	  ○ The superior landmark: top of shoulders
	  ○ Inferior landmark: base of the diaphragm

Pitch	variable, dependent on scanner type.
Tube voltage	120 kVp
Tube current	Use auto mA for chest acquisition: Goal of 40 or 45 mAS depending on BMI: • For BMI<30 kg/m^2: 40 mAS • For BMI≥30 kg/m^2: 45 mAS *Note: ➢ For Siemens and Philips cameras, set reference mAS to goal mAS above. ➢ For GE cameras, set maximum and minimum to +5% and -5%, respectively, to goal mAS above IMPORTANT: In the event that a patient has hardware such as a pacemaker, do not use auto mA. Instead, set goal effective mAS to 40. Replicate tube current used for subsequent scans.
Scan FOV	Large
Display FOV	Unique to subject size
Slice Thickness (collimation)	3mm

Reconstruction Interval / Increment (distance between adjacent slice locations)	3mm
Gap (slice spacing)	None (i.e. contiguous)

Chest – PET Scan

Subject Orientation:	• Same as Chest CT Scan

Scan Locations/Coverage:	• Entire chest
	  ○ The superior landmark: top of shoulders
	  ○ Inferior landmark: base of the diaphragm

Data Acquisition Mode	• 3D
Scan FOV	Large
Display FOV	Unique to subject size
Slice Thickness (collimation)	3 mm
Matrix Size	128 X 128, plus 256 X 256 (or in some cases, another matrix size that is optimized for your scanner type. If 256 X 256 matrix size is not available, please consult with the Imaging Core)
Scan time	5 min per bed position
Gap (slice spacing)	None (i.e. contiguous)
Reconstruction	• Use your standard OSEM construction for your scanner and ensure SAME RECONSTRUCTION PROTOCOL IS USED EACH TIME • Attenuation-correct all images • Correct all projection data for non-uniformity of detector responses, dead time, random coincidences, and scattered radiation. • In plane resolution should be better than 5mm FWHM.

1.2 PET/CT Neck

Neck – CT Scan

Subject Orientation:	• Supine position • **Arms down fully extended with palms resting on anterior thighs, with minimal/no flexion of the elbows.** • With the subject in the supine position, place the neck in a neck holder. Keep the neck straight and be sure that it is not extended or flexed. • Use the axial laser across the orbital meatal line to ensure that there is no rotation of the head to either side. • Velcro straps and/or tape may also be used to maintain subject positioning.

Scan Locations/Coverage:	• Entire neck
	  ○ The superior landmark: Top of the eyes
	  ○ Inferior landmark: sternal notch

Pitch	variable, dependent on scanner type.
Tube voltage	120 kVp
Tube current	Use auto mA for neck acquisition: Goal of 40 or 45 mAS depending on BMI: • For BMI<30 kg/m^2: 40 mAS • For BMI≥30 kg/m^2: 45 mAS *Note: ➢ For Siemens and Philips cameras, set reference mAS to goal mAS above. ➢ For GE cameras, set maximum and minimum to +5% and -5%, respectively, to goal mAS above IMPORTANT: In the event that a patient has hardware such as a pacemaker, do not use auto mA. Instead, set goal effective mAS to 40. • Replicate tube current used for subsequent scans.
Scan FOV	Large
Display FOV	Unique to subject size
Slice Thickness (collimation)	3mm
Reconstruction Interval / Increment (distance between adjacent slice locations)	3mm

Gap (slice spacing)	None (i.e. contiguous)

Neck – PET Scan

Subject Orientation:	• Same as Neck CT Scan
Scan Locations/Coverage:	○ Entire neck ○ The superior landmark: Top of the eyes ○ Inferior landmark: sternal notch
Data Acquisition Mode	• 3D
Scan FOV	Large
Display FOV	Unique to subject size
Slice Thickness (collimation)	3 mm
Matrix Size	128 X 128, plus 256 X 256 (or in some cases, another matrix size that is optimized for your scanner type. If 256 X 256 matrix size is not available, please consult with the Imaging Core)
Scan time	5 min per bed position
Gap (slice spacing)	None (i.e. contiguous)
Reconstruction	• Use your standard OSEM construction for your scanner and ensure SAME RECONSTRUCTION PROTOCOL IS USED EACH TIME • Attenuation-correct all images • Correct all projection data for non-uniformity of detector responses, dead time, random coincidences, and scattered radiation. • In plane resolution should be better than 5mm FWHM.

1.3 Joints PET/CT

CT Scan – Joints: above shoulders to toes

Subject Orientation:	• Supine position • **Arms down, fully extended with palms resting on anterior thighs, with minimal/no flexion of the elbows. Please see pictures below.** • Large restraint (immobilizer, usually with Velcro straps) across the forearms including elbows and hands. • Use the sagittal laser and the subject's nipples as a landmark to ensure that there is no rotation of the chest to either side. • Quiet breathing • Another large restraint across the knees. Feet to be positioned adjacent to each other (pigeon toe), wrapped with a sheet and covered with paper tape. • Subjects should also remove rings and other accessories on hands and feet.
Scan Locations/Coverage:	• From ~4cm above shoulders to toes
Pitch	variable, dependent on scanner type.
Tube voltage	120 kVp

Tube current	• Use fixed mA of 40 mAs
	• Goal: effective mAs = 40
	  ○ Effective mAs = Average mA * Rotation Time/Pitch
	  ○ Exact parameters will vary by scanner manufacturer and model, adjust Auto mA settings accordingly to achieve our goal
	• Use fixed mA for subsequent scans.

Scan FOV	Large
Display FOV	Unique to subject size
Slice Thickness (collimation)	4mm
Reconstruction Interval / Increment (distance between adjacent slice locations)	4mm
Gap (slice spacing)	None (i.e. contiguous)

Positioning Images for PET/CT of Joints:

Pigeon Toe Position of Feet, Covered with Sheet & Paper Tape

PET Scan – Joints: above shoulders to toes

Subject Orientation:	• Same as Joints CT Scan
Scan Locations/Coverage:	• From ~4cm above the shoulders to the toes
Data Acquisition Mode	• 3D
Scan FOV	Large
Display FOV	Unique to subject size
Slice Thickness (collimation)	4 mm
Matrix Size	128 X 128, plus 256 X 256 (or in some cases, another matrix size that is optimized for your scanner type. If 256 X 256 matrix size is not available, please consult with the Imaging Core)

Scan time	• Scan time per bed position is determined by the subject size:
	  ○ **2 .0 min per bed position for BMI<30 kg/m²**
	  ○ **2.5 min per bed position for BMI≥30 kg/m² (but may depend on scanner type)**
	Replicate scan time per bed position for subsequent scans.

Gap (slice spacing)	None (i.e. contiguous)
Reconstruction	• Use your standard OSEM construction for your scanner and ensure SAME RECONSTRUCTION PROTOCOL IS USED EACH TIME • Attenuation-correct all images • Correct all projection data for non-uniformity of detector responses, dead time, random coincidences, and scattered radiation. • In plane resolution should be better than 5mm FWHM.

1.4 IMAGE ACQUISITION GUIDELINES, POST-IMAGING

- **Remove the subject from the scanner and encourage him/her to use the bathroom and empty the bladder.**
- **The subject should be instructed to drink fluids and void frequently throughout the remainder of the day.**

Digital Image Transfer only:

- **The images must be contiguous (no gaps).**
- **Images should be in DICOM format**
- ***All images must be in an uncompressed format*.**
- **Transfer:**

1) attenuation corrected PET data reconstructed with 128X128 Matrix, (neck, chest, and joints) plus 256 X 256 (or in some cases, another matrix size that is optimized for your scanner type. If 256 X 256 matrix size is not available, please consult with the Imaging Core)

2) non-corrected PET data, reconstructed with 128X128 Matrix, (neck, chest, and joints) plus 256 X 256 (or in some cases, another matrix size that is optimized for your scanner type. If 256 X 256 matrix size is not available, please consult with the Imaging Core)

3) CT-AC scans, (neck, chest, and joints)

4) Dose report

- **Images (including raw/original data) should remain digitally archived at the site throughout the entirety of the clinical trial.**
    - **Retain raw data on the scanner until confirmation of acceptable data has been received**
    - **Keep a source disc (CD or DVD) containing images at the clinical site**
    - **Prepare images for transfer to imaging core lab**
- **See 'Image Submission Guidelines' for further instructions**

1.5 safety read for incidental findings

A safety read for incidental findings in the whole body scan is to be conducted by a local radiologist within **72 hours of image acquisition** and the report submitted to the clinical investigative site immediately thereafter. Local radiologists should look for findings that might be of clinical significance that should be shared with the site investigator – e.g., increased focal uptake of tracer that may

represent infectious, immune or malignant processes, or any other pattern of uptake that may cause clinical concern. The report should be provided to the clinical site involved in the TARGET Trial within 72 hours, as any incidental findings may impact TARGET eligibility.

1.6 Rescanning Guidelines

Occasionally, the quality of the scans will be inadequate to provide quantitative data and consideration will be given to re-scanning. The Imaging Core will ALWAYS determine the need for a repeat scan; the decision to repeat a scan should never be determined by the local imaging site. See Section 2.5.d. for more information about quality assessment.

- **Baseline Visit**: The only time that a repeat scan may be requested by the Imaging Core is if both vascular scans (neck and chest) at the Baseline visit are inadequate to provide quantitative data. This determination will be made by the Imaging Core within 48 hours of submission of the scan, and a request for a repeat scan(s) will be made by the Imaging Core to the local imaging site. If a patient had a repeat scan(s) at the Baseline visit, the following protocol must be followed at the Month 6 follow-up scan session:
    - **No whole body scan will be performed**
    - **Only one vascular (neck or chest) scan will be performed**. The scan type will be determined by the Imaging Core. Only the vascular scan that provided adequate (or higher quality) quantitative data at the Baseline visit will be performed at Month 6 visit. The local imaging site will be informed by the Imaging Core, in advance of the patient's appointment, as to which scan (neck or chest) to perform.

- **Month 6 Visit:** In patients who had a technically adequate Baseline Scan (1 Neck, 1 Chest, and 1 Whole Body acquisition) but had a technically inadequate Month 6 vascular scan (either chest or neck), as determined by the Imaging Core, a repeat scan may be requested. In this case repeat scanning will be requested of **either the neck, the chest, or both**. As above, the **whole body scan should never be repeated at this visit**, regardless of quality.

2. Site training, Image Qualification and Image Submission Guidelines

2.0 Introduction

This Site Qualification and Image Submission Guidelines document is provided by the Icahn School of Medicine- Massachusetts General Hospital Imaging Core Laboratory for the TARGET Trial (ICL). It is intended for Imaging Centers participating in TARGET and is designed to provide detailed information regarding the process for qualifying an Investigator Site for participation in the imaging component of TARGET and all of the accompanying start-up procedures.

Items such as imaging training, image submission, query response and resolution, and study design will be covered in these guidelines. These guidelines will used as a supplement to regular procedures already in place at qualifying sites. For details regarding the imaging protocols, please refer to Image Acquisition Guidelines (also provided).

Each site must be qualified before submitting study exams as this process ensures that sites are capable of following study protocol. Careful and well-documented training of all staff is also required before imaging may begin at the clinical site. Details regarding Site Qualification are discussed in Section 2.3.

2.1 Study Design

The objective of this Imaging Study is to determine vascular inflammation in subjects by implementing 18F-fluorodeoxyglucose Positron Emission Tomography/Computed Tomography (FDG-PET/CT) imaging, to subjects participating in this trial.

2.2. Definitions

Imaging Core Lab (ICL): A collaboration between the Icahn School of Medicine and Massachusetts General Hospital Imaging Core Laboratories : Centralized coordination facility for all procedures associated with PET/CT image acquisition, processing and review. The facility is responsible for receiving, tracking, and archiving PET/CT images that are associated with this study. The Imaging Core Lab also performs quality control (QC) checks on all PET/CT examinations and related documentation received. PET/CT images are checked for consistency, completeness and protocol compliance.

Investigator Sites: The institutions responsible for enrolling clinical study subjects and obtaining clinical data associated with study subjects. The Investigator Site is also responsible for managing imaging scheduling and for preparing and forwarding images acquired to the ICL, as per protocol. In addition, they are responsible to ensure responses to any queries generated.

Imaging Centers: These centers are generally either a physical part of the main Investigator Site, or function as a satellite site, and are responsible for acquiring images associated with clinical study subjects. PET/CT images will be obtained according to the study protocol and the study Image Acquisition Guidelines provided.

2.3 Site Qualification Procedures

2.3.a. Site Contact Information

Investigator Sites will provide ICL with a list of contact information for study staff (Principal Investigator and Study Coordinator). This listing will be sent on a weekly basis during start-up phase, and on an as-needed basis thereafter.

2.3.b. Imaging Surveys

ICL will be responsible for distributing Imaging Surveys. Imaging surveys collect contact information from the technologists at the Imaging Centers and identify the types of scanners, scanning capabilities, and data transfer capabilities available at the sites. Instructions regarding the completion of the imaging survey will be sent by ICL to the primary contact at each imaging center.

ICL will download reports from the online survey system on a weekly basis. All completed records of site contact information and Imaging Surveys will be filed in a site folder on the study-specific Master Study File. Imaging Survey responses will be reviewed on weekly basis by a Clinical Research Associate (CRA) for completeness and compatibility with the core lab systems. If any information is missing or

incorrect, the CRA will follow up with the Investigator Site to resolve the issue. Any issues regarding the site's equipment or abilities to comply with the imaging protocol will be raised by ICL to the Investigator Site.

2.3.c. Site Initiation Tracking

The core lab CRA will create a study-specific ***Site Qualification Spreadsheet***, stored on an electronic file to track the status of all qualification steps for each Imaging Center participating in the study. Upon completion of each of the tasks described in this section, the spreadsheet will be updated to show the dates that sites completed each step in site initiation. The Imaging Center name and contact information provided by Sponsor will also be entered into the spreadsheet, along with the clinical site PI(s) associated with each Imaging Center.

2.3.d. Site Radiation Safety Officer/Committee Attestation

The Radiation Safety Officer or Commitee at each imaging site must sign the attestion form at the beginning of this protocol to state that they have reviewed this Imaging Protocol and that they agree with the Radiation Safety Monitoring Plan. This must be completed before trial imaging commences.

2.4 Study Imaging Binder

The ICL will provide the imaging centers with forms to be kept in a ***Study Imaging Binder at their site*** for PET/CT imaging. Please note that the ICL will not be providing a physical binder. These forms should be kept at the imaging site. Below is a description of these materials:

- The **Image Acquisition Guidelines (IAG)** are generated by the ICL in an effort to standardize the imaging associated with the clinical trial. The standardized guidelines are distributed to each Imaging Facility and details the imaging protocol.

- The **PET Adjunctive Data Forms (ADFs)** is to be completed and signed by the person submitting images. Complete ADFs can be transmitted via email at the time images are submitted.

- The **Image Delivery Receipt** is an automatically generated e-mail sent from the ICL sFTP site notifying the sender that delivery of images was successful. The delivery receipt lists the item downloaded, the download date and time, recipient, and transferred file size. An example of a delivery receipt is included in the Sample Forms tab of the Study Imaging Binder. The binder also includes a tab for storage of Image Delivery Receipts that are received for subjects participating in the trial.

- The **Case Acceptance Notification (CAN)** is sent from ICL to the primary contacts at the Investigator Site and Imaging Center. This form serves to inform the study staff that the entire PET/CT image set and IRF for a subject have been received and checked for completeness and acceptable quality by the ICL staff. A sample of the CAN is provided in the Study Imaging Binder. The binder also includes a tab for storage of CANs that are received for subjects participating in the trial.

- The **Case Query Notification (CQN)** is sent from ICL to the primary point of contact at the Investigator Site and the Imaging Center within 2 business days of receipt of an unacceptable/incomplete PET/CT dataset. The form is sent via e-mail and will describe the query and indicate the action(s) required to be taken towards submission resolution of the issue described. A sample of the CQN is provided in the Study Imaging Binder. The binder also includes a tab for storage of CQNs that are received for subjects participating in the trial.

2.5 Operations Summary

The following is an overview of operational activities relating to the trial. This section describes the activities at the Investigator Sites and the ICL, which includes review by an independent, central reader.

For all image transfers, ICL sFTP System will be used. A log-in and password to this system will be provided to the primary contact at each imaging center, generally a lead technologist, unless an alternate point of contact is designated by the site. Detailed instructions on submitting data via the sFTP system can be found in Section 2.6 of this document.

2.5.a. PET/CT Phantom Image Data Transfer

Each Imaging Center will be required to submit image sets of standardized PET/CT phantoms prior to approval for imaging. An American College of Radiology (ACR) phantom produced during the most recent quarterly assessment performed at the Imaging Center would suffice.

Initial phantom data will undergo a preliminary quality control (QC) review by the imaging CRA to ensure that images transferred are in proper format, that both PET and CT series are present, and the images appear to be of acceptable quality. Initial phantom datasets will then be forwarded from the Osirix workstation to the Reader workstation for quantitative analysis. ICL CRA will e-mail the reader that the data has been sent and is available for review. The reader will reply via e-mail within 3 business days with the results of quantitative analysis of the phantom data. Results of the review should be in binary form: either positive (pass) or negative (fail). Additional recommendations on how to achieve auto mA based on your scanner may be provided after submission of the phantom images.

Failure to meet any of the criteria above, or a negative review by the reader, will result in a query to the site. If the initial phantom dataset received from a site is determined by the Reader to not be of acceptable quality, ICL will request a rescan of phantom data and note the parameters described in the Image Acquisition Guidelines that should be adjusted to optimize the quality. The date of query sent and reason for query are tracked in RIS and are associated to the specific phantom data timepoint from which they originated. In cases where queries are necessary, the CRA will NOT archive the study to ICL PACS and instead select “No Image” when prompted to link to a dataset PACS. After tracking is complete, the RIS record can be closed.

Upon determination that the dataset is acceptable, data will be archived to ICL PACS and associated with the corresponding timepoint record in RIS.

The study-specific site qualification spreadsheet is updated to indicate the date of receipt of phantom data transfer.

Once an imaging center has been approved, all subsequently received quarterly phantom data will undergo QC by the imaging CRA only. Subsequent phantom images are permitted to be done under the normal quality assurance (QA) procedures and protocols in place at the Imaging Center. Processing of these phantom scans will follow the procedures described in this section. Subsequent phantom datasets from sites can be entered under the same subject in RIS as an additional timepoint.

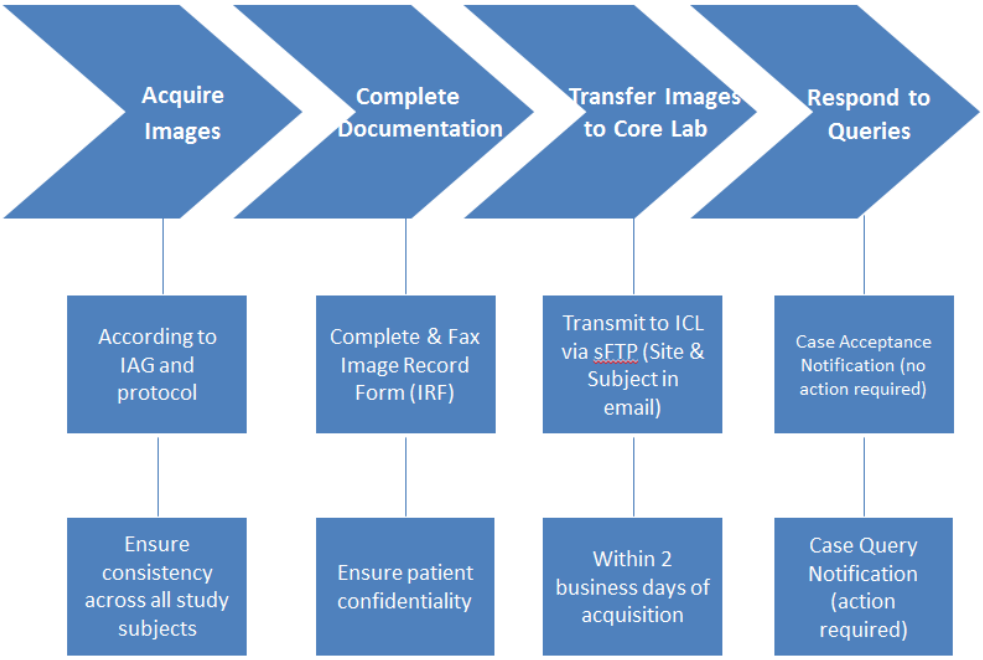
2.5.b. Imaging Training

To ensure consistency in scan acquisitions across all imaging centers, a primary technologist will be appropriately trained by ICL on all relevant aspects of this study prior to scanning study subjects. The participating Imaging Center is also encouraged to identify a back-up technologist who will undergo the same training. ICL will require PET/CT Technologists to participate in an online video training prior to the onset of the study. Additional Site personnel will also be invited and are encouraged, to attend the training session.

Training will include reading the Imaging Protocol, watching a training video, and attending a Question & Answer teleconference with personnel from your assigned Imaging Core Lab (Mount Sinai or MGH). ICL will provide login information prior to all training sessions.

2.5.c. Imaging and Data Collection

The figure below summarizes the expectations of the Investigator Site and/or Imaging Center in support of the imaging component of the trial. Following the figure are detailed instructions for imaging data collection.



1. The Investigator Site will refer subjects to the Imaging Center for PET/CT imaging exams. All imaging exams must be conducted by trained Technologists in accordance with the Image Acquisition Guidelines.

2. The Investigator Site, or a designee in the Imaging Center, will initiate PET Adjunctive Data Forms (ADFs) for each subject to be imaged. The ADFs are to be completed and signed by the person submitting images. ADFs can be transmitted via fax or email to ICL (contact details will be provided).

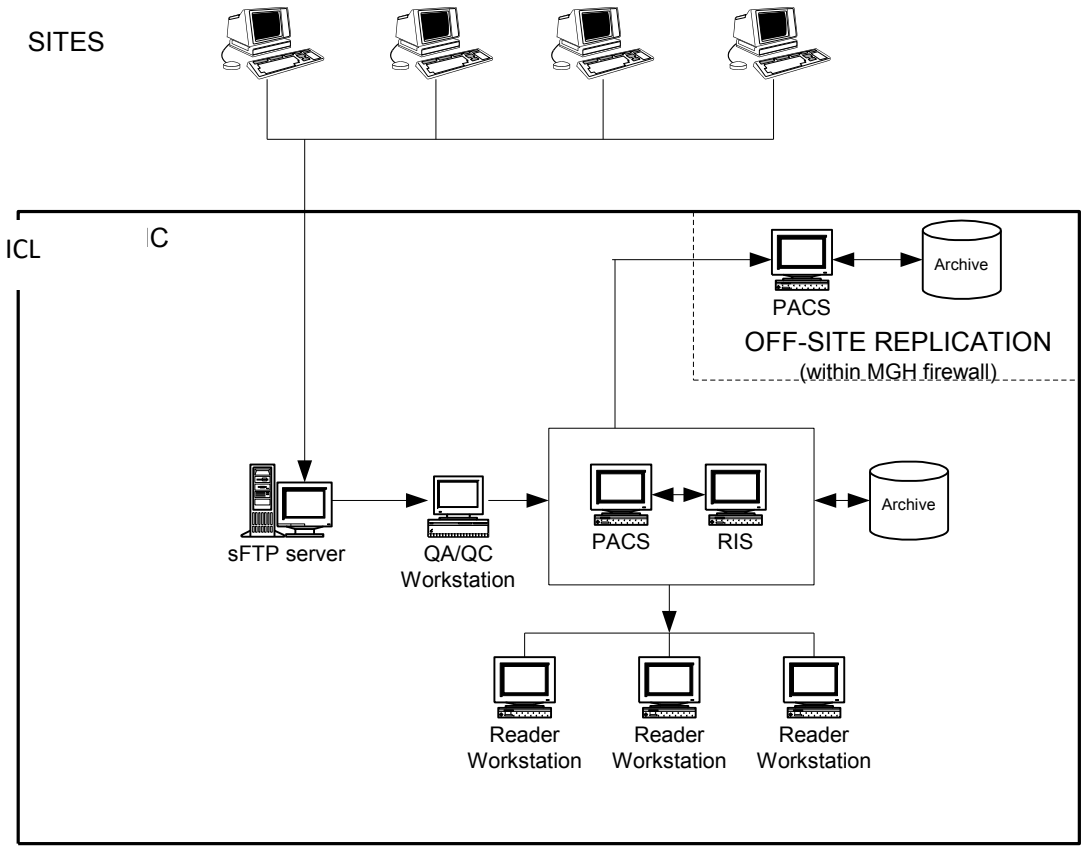
3. Images must be anonymized prior to submission to ICL according to the following guidelines:

    i. Subject Name replaced with Study ID (Site (xx digit) – Subject (xx digit))
    ii. Year of birth should remain, if permitted by local regulation, and serve as a key identifier for the record
    iii. Date of Scan must be included on the image
    iv. Medical Record number replaced with a 7-digit standard code of zeros (e.g. 0000000)
    v. Any other protected health information (PHI) removed from any additional fields

4. The complete and anonymized PET/CT images will be sent to ICL via secure File Transfer Protocol (sFTP). See Section 2.6: sFTP Data Transfer Instructions for details regarding submission of images. All images must be submitted in DICOM format. Images should be sent *on the day of acquisition, or within 2 business days* should it not be possible to send on same day. If it is not possible to transfer the images within that time frame and/or if there are any technical difficulties in transferring images, please alert ICL via e-mail or phone.

    Specifically, invitations will be sent to imaging sites allowing external users to utilize a web browser to submit data to the ICL FTP site. The data is received at ICL, after which the data is sent

to a RIS/PACS (Radiological Information System/Picture Archiving and Communication System). This technical infrastructure is displayed in the graphic below.



5. After PET/CT images are received at ICL, a Clinical Research Associate (CRA) will confirm image set completeness and proper labeling/anonymization of images. Subject and timepoint information will be verified on the Image Record Form. A quality control (QC) will be performed to verify that the Image Acquisition Guidelines were followed. If the imaging associate finds an artifact that could impair the ability to interpret images, the nature of the artifact will be discussed with ICL medical staff before being escalated to external study personnel.

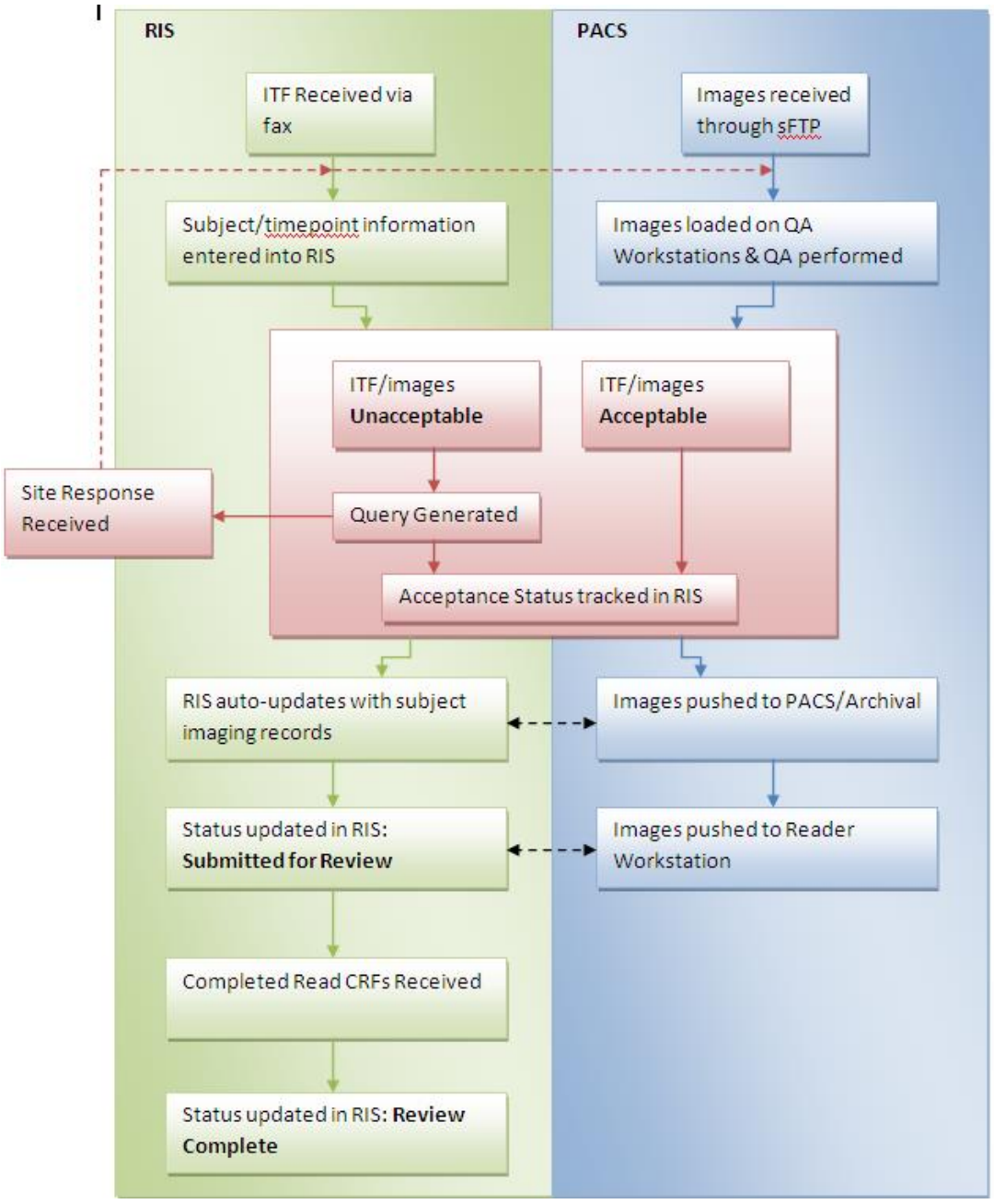
6. ICL will notify the Investigator Site and Imaging Centers of the results of the QC of each image set submitted in the form of either a Case Acceptance or a Case Query Notification.

7. The Case Acceptance Notification informs the Study Coordinator and Imaging Center that PET/CT imaging for the timepoint is complete and of acceptable quality. Any deviation from the guidelines should be discussed with ICL in advance. Any change in parameters must be pre-approved and noted on the Image Record Forms.

8. If a Case Query Notification is issued, the notification will indicate actions that need to be taken by the Imaging Center. The site is required to resolve the query and/or send the missing/discrepant information to ICL within 2 business days. This short turnaround time is necessary due to the time-sensitive nature of some imaging visits. If resolution of the query cannot be completed, the site will be required to notify ICL (via email) within the two-business day time frame. This notification must include a definitive date for query resolution.

Once the query has been resolved, a Case Acceptance Notification will be issued to notify the Investigator Site and Imaging Center that the images are now complete and of acceptable quality.

9. Flow of subject information from clinical sites and corresponding images is shown in the figure below.



2.5.d Data and Image Quality Evaluation

(A) Data quality will include evaluation of CRF data as well as image quality. CRF data will be used to assess adherence to the protocol. Data evaluated to assess adherence to the imaging protocol will include: i) tracer circulation time (ii) intra-subject difference in circulation time (between scans) (iii) fasting blood glucose (iv) injected minus residual activity, iv) scanner used, v) arm position, vi) reconstruction method, vii) time per bed position, viii) acquisition mode, ix) slice thickness as well as other characteristics provided in the CRF.

(B) Image Quality Images will be graded as: 1 (Excellent), 2 (Marginal), and 3 (Unacceptable) in each of the three vessel beds. An example of a marginal image set is one where it was deemed difficult to derive measurements, whereby some confidence in the measured values is lost. An example of an unacceptable image set is one where image quality precludes measuring the image (e.g. excess motion, artifact, spill-over). Data analysis will be performed first on data derived from Excellent quality scans only, then Excellent and Marginal quality scans combined.

2.5.e Radiation Dose Monitoring

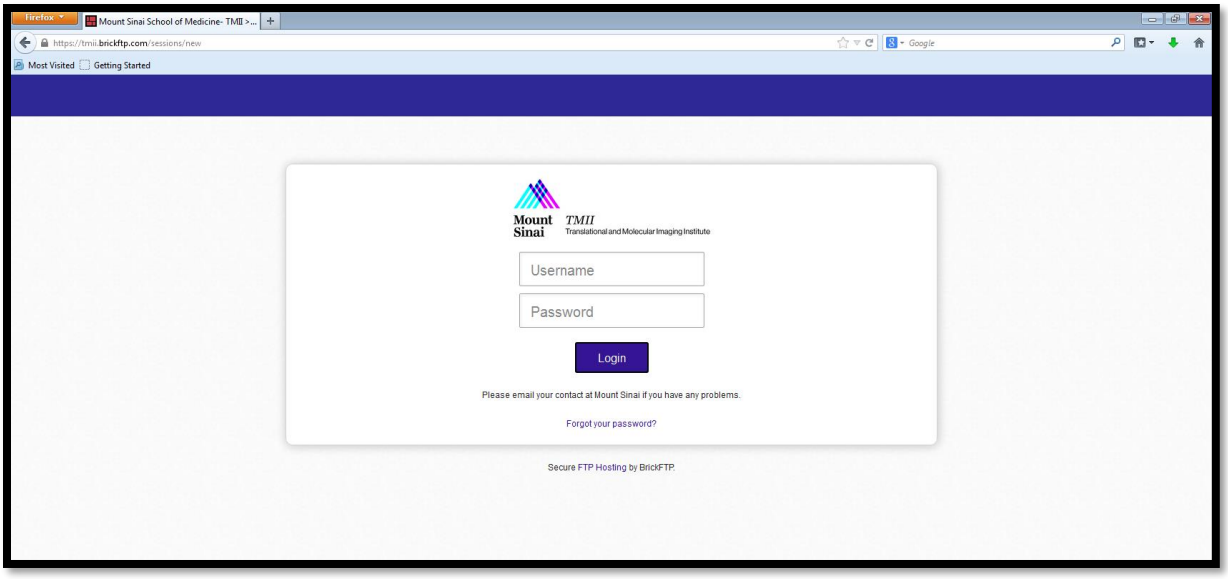
Radiation exposure for each subject will be monitored by the imaging core lab, the IRB, and the DSMB. Please see Appendix D: Radiation Safety Monitoring Plan (RSMP) for more details.

2.6 sFTP Data Transfer Instructions

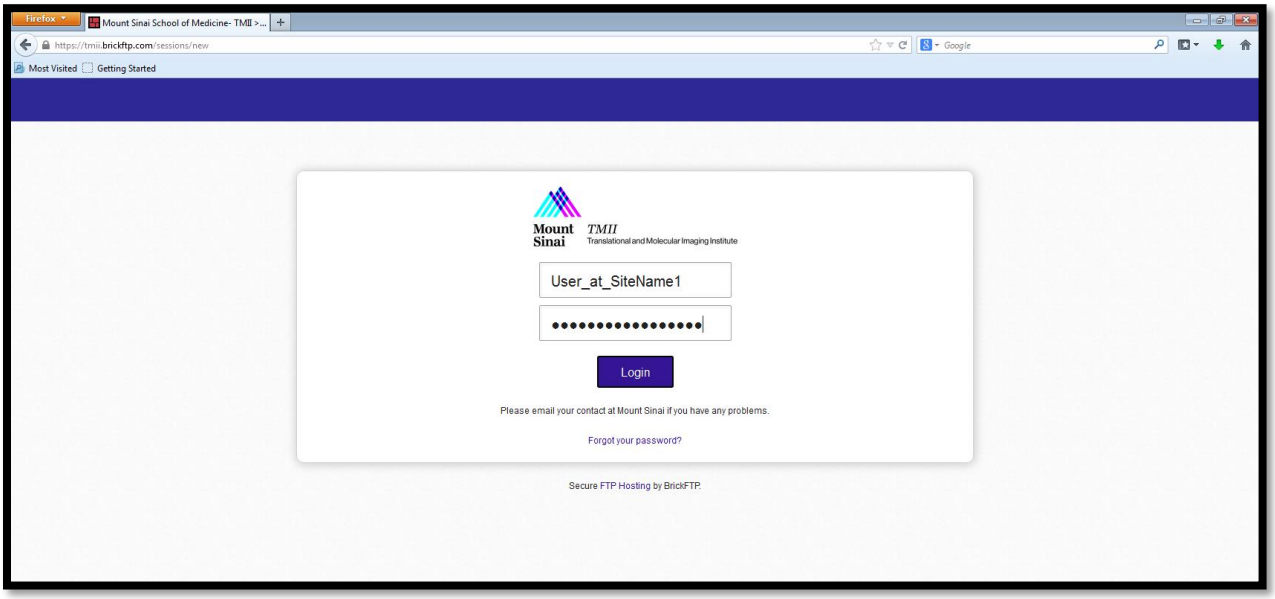
The copy of data for submission can be transferred to ICL over a secure FTP server. The steps for transferring the data via secure FTP are different depending on your Core Lab assignment (Mount Sinai or MGH):

For Sites Assigned to the Mount Sinai Core Lab:

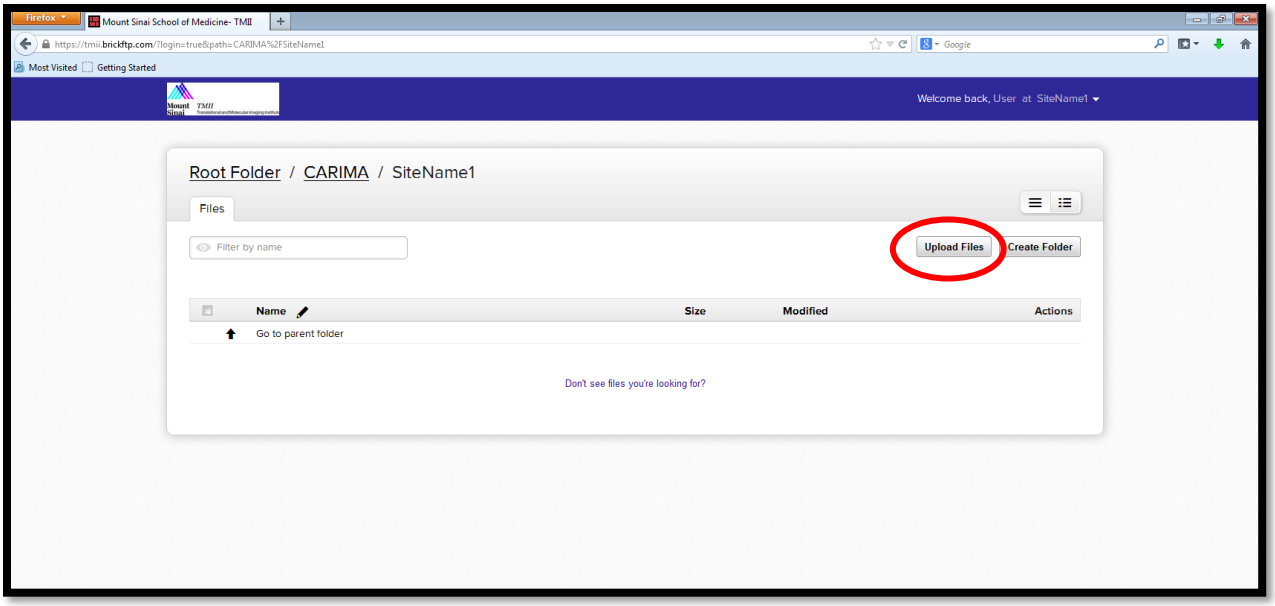
1) All DICOM Images should be copied into single folder. Set the folder name as the patient ID.
2) Zip or compress the patient data folder.
3) Type https://tmii.brickftp.com into a browser window.



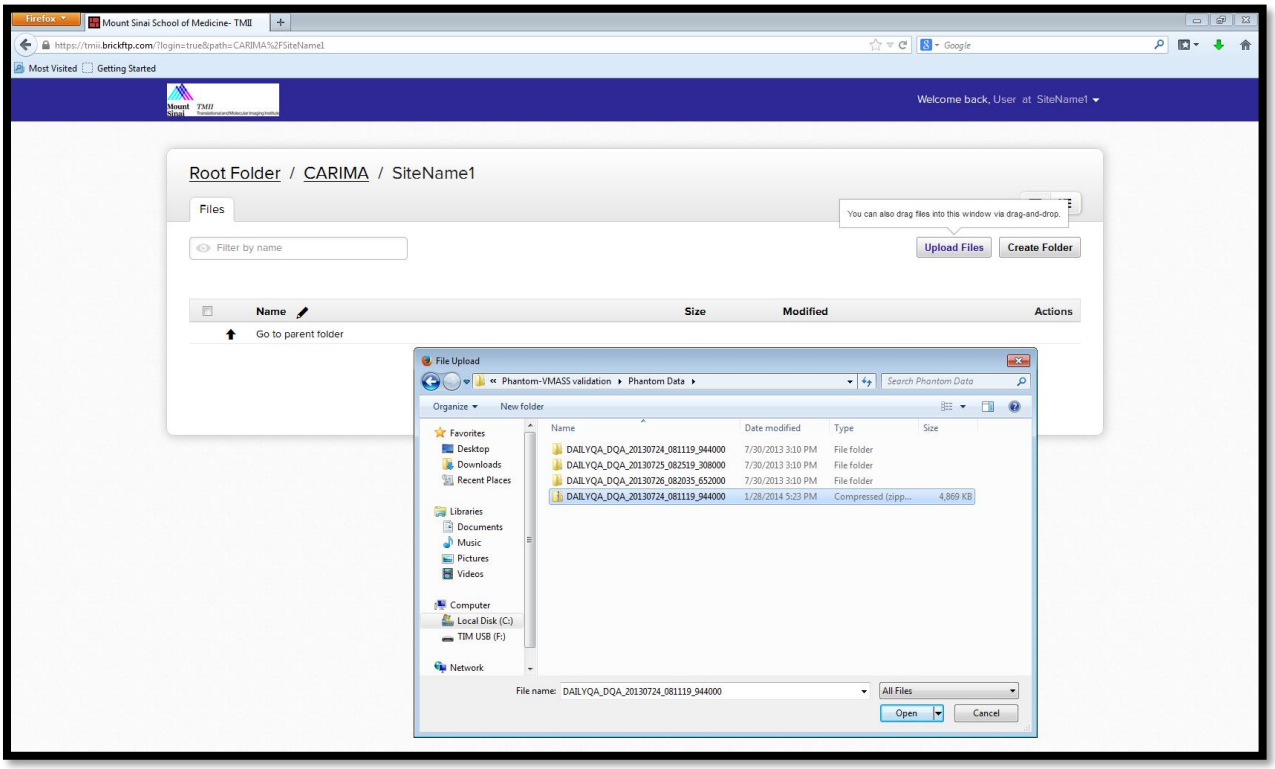
4) Log in using your site-specific username and password (will be provided to you by ICL).



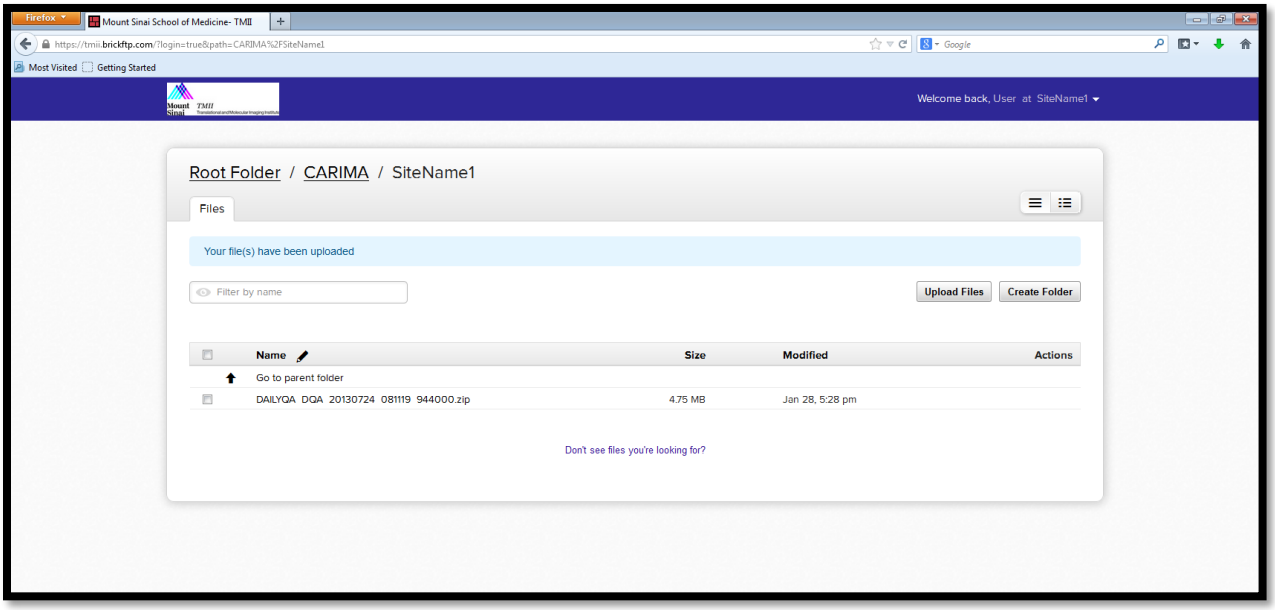
5) Click on the “Upload Files” button.



6) Select the compressed or zipped patient data folder and click “Open”.



7) The zipped patient data folder will be visible in the browser once the data has been uploaded.



For Sites Assigned to the MGH Core Lab:

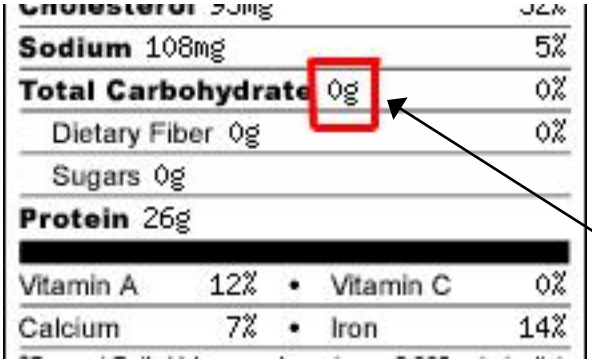
1. Create a folder on your desktop
2. Copy DICOM data from CD into the desktop folder
3. Right click folder > Send To > Compressed (zipped) folder
4. Log in to the system : https://transfer.partners.org/courier/web/1000@/wmLogin.html
5. Select Send File tab
6. Enter < mitc@partners.org > into the To field
7. Select Choose File > browse for the zipped file on your desktop
8. Add any notes pertaining to the scan or assessment (optional)
9. Select notification preferences from the Additional Options field (optional)
10. Click Send
11. Click OK after receiving confirmation that the files have been sent

ATTACHMENT A. DIETARY INSTRUCTIONS FOR SUBJECTS

- **It is essential that the subjects follow a low-carbohydrate diet starting with the evening meal or 5 pm (whichever comes first) on the evening before imaging, and that they refrain from exercise for 24 hours prior to imaging.**

  <u>After 5:00pm on the day before the imaging study:</u>
  Eat and drink **ONLY** the food listed below:
  - Meat
  - Fish
  - Eggs
  - Green Vegetables
  - Soy
  - Cheese (small amounts)
  - Water (no soda, fruit juice, or alcoholic beverages)
  - Coffee or tea (as long as no milk or sugar is added)

  Be careful to **avoid** the food listed below:
  - Fruit
  - Bread (including muffins, crackers, and pastries)
  - Cereal
  - Pasta
  - Rice
  - Potatoes, Carrots, and Corn
  - Salad Dressing with added sugar or sweetener
  - Candy
  - Milk and yogurt



When in doubt, check the nutrition label!

Keep this number *as low as possible.*

On the day of the imaging study:

- **No** carbohydrates (bread, cereal, milk, juice, coffee with cream, etc). Water or coffee/tea (without sugar or milk) is ok.
- Morning Imaging: Subjects being imaged in the morning must have fasted for at **least 10 hours the previous night.**
- Afternoon Imaging: Subjects being imaged in the **afternoon must have fasted for at least 10 hours** the previous night but may have a breakfast from the recommended menu above and then **fast for at least 4 hours** prior to imaging appointment.
- Diabetics are permitted to take their medications on the morning of the imaging.