

REVIEW ARTICLE

Testing the Effects of Disease-Modifying Antirheumatic Drugs on Vascular Inflammation in Rheumatoid Arthritis: Rationale and Design of the TARGET Trial

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Individuals with rheumatoid arthritis (RA) are at increased risk for atherosclerotic cardiovascular disease (ASCVD) events relative to the general population, potentially mediated by atherosclerotic plaques that are more inflamed and rupture prone. We sought to address whether RA immunomodulators reduce vascular inflammation, thereby reducing ASCVD risk, and whether such reduction depends on the type of immunomodulator. The TARGET (Treatments Against RA and Effect on 18-Fluorodeoxyglucose [¹⁸F-FDG] Positron Emission Tomography [PET]/Computed Tomography [CT]) trial (NCT02374021) will enroll 150 patients with RA with active disease and an inadequate response to methotrexate. Participants will be randomized to add either a tumor necrosis factor (TNF) inhibitor (etanercept or adalimumab) or sulfasalazine and hydroxychloroquine to their background methotrexate. Participants will undergo full-body ¹⁸F-FDG-labelled PET scanning at baseline and after 6 months. Efficacy and safety evaluations will occur every 6 weeks, with therapy modified in a treat-to-target approach. The primary outcome is the comparison of change in arterial inflammation in the wall of the aorta and carotid arteries between the randomized treatment groups, specifically, the change in the mean of the maximum target-to-background ratio of arterial ¹⁸F-FDG uptake in the most diseased segment of either the aorta and carotid arteries. A secondary analysis will compare the effects of achieving low disease activity or remission with those of moderate to high disease activity on vascular inflammation. The TARGET trial will test, for the first time, whether RA treatments reduce arterial inflammation and whether such reduction differs according to treatment strategy with either TNF inhibitors or a combination of nonbiologic disease-modifying antirheumatic drugs.

INTRODUCTION

Individuals with rheumatoid arthritis (RA) are more likely to experience events related to atherosclerotic cardiovascular disease (ASCVD) compared with an otherwise similar person without RA. Overall, rates of myocardial infarction (MI) and death from ASCVD are 50% higher for populations with RA compared with

control populations (1,2). ASCVD is the largest contributor to excess mortality in RA (3), resulting in a reduced life expectancy of at least 6 to 7 years (4). As RA is the most common autoimmune rheumatic disease, with a prevalence of approximately 1% of adults worldwide, this magnitude of excess ASCVD represents a substantial public health problem, with an estimated direct cost of \$500 million per year in the United States alone (5). Although

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rates of ASCVD events appear to be declining in RA, rates have also been declining in the general population, resulting in a continued ASCVD gap between RA and non-RA populations (6). Excess events appear to be mediated by both an increase in the overall burden of atherosclerosis (7,8) and a shift in the composition of atherosclerotic plaques toward those that are more inflamed and rupture prone (9,10). Increased ASCVD risk is observed at all phases of RA (ie, early versus established) (6), and more ASCVD events than expected have even been reported in the years preceding the onset of the signs and symptoms of RA (11), possibly reflecting increased preclinical systemic inflammation and/or autoimmunity (12).

Although traditional ASCVD risk factors, such as hypertension, smoking, and diabetes, are contributors to ASCVD in RA (13), they alone do not account for all of the excess risk (14). Additional potential contributors are the inflammatory cytokines (eg, tumor necrosis factor [TNF], IL-6, and IL-1 α), chemokines, and other inflammatory mediators that are characteristic of RA pathobiology (15). In RA, these circulate in high concentrations, with the potential to exert effects on tissues outside the synovium, such as the arterial wall. These same mediators are well-established drivers of atherogenesis, plaque instability, and atherothrombosis in the general population (16).

THE EFFECT OF RA THERAPIES ON ATHEROSCLEROSIS IS UNCERTAIN

Although a decline in ASCVD rates among patients with RA has generally paralleled the increasing adoption of “treat-to-target” and, more recently, “treat-to-remission” therapeutic approaches (6), only an ecological correlation between aggressive RA management and ASCVD reduction can be inferred from these trends. The only randomized clinical trial conducted to test the comparative efficacy of RA immunomodulators on ASCVD events demonstrated that the IL-6 inhibitor tocilizumab was noninferior to the TNF inhibitor etanercept in the development of incident major adverse cardiovascular events over an average of 3.2 years (17). However, the trial was not designed to explore whether the drugs are protective against ASCVD or whether they merely have a similar neutral effect. Other than this single trial, the combined findings from a number of observational cohort studies provide a strong argument for certain immunomodulator treatments being associated with lower ASCVD event rates (18). The majority of these studies have focused on either methotrexate (MTX), the most commonly used nonbiologic disease-modifying antirheumatic drug (DMARD), or TNF inhibitors (TNFis), the most commonly used anticytokine biologic DMARD class. In meta-analyses (18), MTX and TNF inhibitor use were both associated with an average reduction of approximately 30% in the hazard of ASCVD events compared with no treatment or alternative DMARDs. However, there was considerable variation in the magnitude of effects across studies, and, in some, no protective effect was observed. Fewer

studies are available for other nonbiologic DMARDs, although a modest protective effect of sulfasalazine (SSZ) was also reported (19). Despite some evidence that hydroxychloroquine (HCQ) has potential antiatherogenic effects (20,21), no studies have demonstrated an ability of HCQ to reduce ASCVD events. Other biologics used in RA have demonstrated similar ASCVD rates compared with TNF inhibitors (17,22), although this has not been addressed definitively.

DMARDs are immunomodulatory, with effects on the activation status of immune effector cells, particularly macrophages, and their expression of inflammatory cytokines and other inflammatory mediators that underlie the pathobiologies of both RA and atherogenesis/atherothrombosis. Multiple lines of evidence suggest that specific DMARDs modulate ASCVD in RA. MTX or TNF inhibitor treatment was associated with an improvement in endothelial function testing (23–25), although the effect was transient in some studies (23). In longitudinal cohort studies, TNF inhibitor treatment was associated with a decline in the progression of carotid plaque (26,27) and with atherosclerotic plaque transition from noncalcified (ie, potentially more rupture prone) to calcified (ie, potentially more stable) status (28). In a trial of patients without RA with prior ASCVD events or high ASCVD risk, the IL-1 inhibitor canakinumab was associated with an 18% reduction in the hazard of ASCVD events compared with placebo (29). However, MTX was not associated with a reduction of ASCVD events among patients without rheumatic disease (30). Taken together, these studies suggest the potential for DMARDs to modify atherosclerotic plaques in a way that may reduce ASCVD risk in RA, but whether this effect differs across DMARDs with differing mechanisms of action and whether the effect parallels other measures of DMARD efficacy, such as the reduction in joint swelling and stiffness, has not been studied experimentally.

CARDIOVASCULAR RISK STRATIFICATION AND IDENTIFICATION OF VASCULAR INFLAMMATION IN PATIENTS WITH RA

Risk stratification tools used to identify those at risk for ASCVD events, such as the Framingham Risk Score and American Heart Association and American College of Cardiology risk algorithms, have been shown to underperform in RA (31). Given this issue, a direct visualization of the arteries of patients with RA is needed to fully assess the efficacy of DMARDs on atherosclerotic plaque, and several possible imaging modalities are available. Carotid ultrasound can be used to measure multiple parameters of the arterial wall that indicate vascular remodeling and atherosclerosis. However, ultrasound is operator dependent and challenging to standardize in the context of a multicenter trial. Quantification of a coronary artery calcium (CAC) score by computed tomography (CT) is a safe, standardized, and relatively inexpensive modality to assess the burden of coronary atherosclerosis. However, the change in CAC in response to interventions does not indicate

clinical efficacy, as important plaque-stabilizing interventions, such as statins, actually associate with an increase in CAC that parallels the transition to less rupture-prone plaque (32). CT angiography can be used to visualize calcified, mixed, and noncalcified plaques but may not be responsive to change over the course of a short trial (33), incurs a considerable amount of radiation exposure, and, perhaps most relevant to RA, does not reveal any direct information about the change in the inflammatory status of atherosclerotic plaques.

Increasingly, 18-fluorodeoxyglucose (^{18}F -FDG) positron emission tomography (PET)/CT imaging is being used to quantify and track the presence and extent of atherosclerotic plaque inflammation in the aorta and carotid arteries. Cellular ^{18}F -FDG uptake reflects the rate of tissue glycolysis, which is higher in areas containing

inflamed tissues. Activated macrophages have relatively high glycolytic rates (34,35) and hence avidly accumulate ^{18}F -FDG (36). Moreover, ^{18}F -FDG uptake in atherosclerotic plaques correlates with the density of macrophages determined histologically (37–41). Higher arterial ^{18}F -FDG uptake was associated with cardiovascular disease events in the general (ie, non-RA) population (42,43), and ASCVD-modifying therapies, such as statins, were associated with a reduction in arterial ^{18}F -FDG uptake in a dose-dependent manner that parallels the expected degree of ASCVD risk reduction observed with these agents (44–46). In a small trial of 17 patients with RA without known ASCVD compared with 34 non-RA control subjects with known ASCVD (10), the RA group had higher aortic wall ^{18}F -FDG uptake. Moreover, aortic wall ^{18}F -FDG uptake decreased by 18% after 8 weeks of uncontrolled open-label treatment with

Table 1. Detailed eligibility criteria

<p>Inclusion (subjects who meet all of the following criteria at screening are eligible for enrollment into the study)</p> <ul style="list-style-type: none"> • Written informed consent signed by the subject • Fulfill ACR/EULAR 2010 criteria for RA • Men ≥ 45 yr old and women ≥ 50 yr old • MTX for ≥ 8 wk at ≥ 15 mg/wk or on at least 7.5 mg/wk for ≥ 8 wk with a documented intolerance of higher MTX doses and stable dose for the previous 4 wk • DAS28 > 3.2 • Able to swallow pills • Men and women with reproductive potential must agree to practice effective measures of birth control • If taking prednisone (or equivalent corticosteroid), the dose must be ≤ 10 mg/d at the time of the baseline ^{18}F-FDG PET/CT scan and must not change by more than ± 3.0 mg for the 4 wk prior to the baseline ^{18}F-FDG PET/CT (if subjects are taking steroids every other day, divide the dose by 2 to evaluate eligibility) • If taking a low- or moderate-intensity statin, the dose must be stable for 6 wk prior to screening and must not change during the 6 mo of the trial • Willing to comply with all study procedures and be available for the duration of the study • RA without psoriasis or with psoriasis if rheumatoid factor $\geq 2 \times$ ULN or anti-CCP $\geq 2 \times$ ULN. <p>Exclusion</p> <ul style="list-style-type: none"> • Use of biologic DMARD or small-molecule DMARD (eg, tofacitinib) in the past 6 months or use of rituximab ever • Nonbiologic DMARDs other than MTX or HCQ for two months prior to Screening • Considered to be an etanercept or adalimumab failure by their primary rheumatologist • Current use of > 10 mg/day prednisone • Current use or use within the previous 12 mo of a high-intensity statin (atorvastatin: ≥ 40 mg; rosuvastatin: ≥ 10 mg) or a PCSK9 inhibitor (alirocumab, evolocumab, or bococizumab) • Prior patient-reported, physician-diagnosed clinical cardiovascular event, including myocardial infarction, angina, stroke, uncompensated or severe heart failure (NYHA class III or IV), and prior vascular procedure (coronary artery angioplasty or stenting, carotid endarterectomy, or 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, or polymyalgia rheumatica • Any of the following other autoimmune and/or chronic inflammatory diseases: inflammatory bowel disease, Crohn disease, cutaneous or systemic lupus, systemic vasculitis, giant cell arteritis, polymyositis, dermatomyositis, sarcoidosis, or scleroderma • Cancer treated in last 5 yr (except basal and squamous cell) or any lymphoma or melanoma • Type I diabetes mellitus or type II diabetes treated with insulin or uncontrolled with HbA1c $\geq 7\%$ from the past 6 mo • Known history of transient ischemic attack, stroke, myocardial infarction, or revascularization for coronary or peripheral artery disease • Known pregnancy, HIV, hepatitis B, hepatitis C, or 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 count $< 3.5 \times 1000$ n/μl, hematocrit $< 30\%$, platelet count $< 90 \times 1000$ n/μl, estimated glomerular filtration rate < 50 ml/min, AST (liver function test) > 60 U/L, or ALT (liver function test) > 84 U/L • Intra-articular injection of corticosteroids within the 4 wk prior to the potential baseline ^{18}F-FDG PET/CT • Two or more of the following high-dose radiation scans: CT scan with contrast, angiogram, SPECT nuclear medicine scan, or myocardial (cardiac) perfusion scan in the past year
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^{18}F -FDG, 18-fluorodeoxyglucose; ACR, American College of Rheumatology; ALT, Alanine transaminase; AST, Aspartate transaminase; CCP, cyclic citrullinated peptide; CT, computed tomography; DAS28, Disease Activity Score 28; DMARD, disease-modifying antirheumatic drug; EULAR, European League Against Rheumatism; G6PD, glucose-6-phosphate dehydrogenase; HbA1c, Hemoglobin A1c; HCQ, hydroxychloroquine; HIV, human immunodeficiency virus; MTX, methotrexate; NYHA, New York Heart Association; PCSK9, Proprotein convertase subtilisin/kexin type 9; PET, positron emission tomography; RA, rheumatoid arthritis; SPECT, single-photon emission computerized tomography; TB, tuberculosis; ULN, upper limit of normal; WBC, white blood cell.

a TNF inhibitor (etanercept or adalimumab). Although small and inconclusive, these results provide preliminary evidence that arterial ^{18}F -FDG is higher in RA and is responsive to change with immunomodulators; together, these findings justify a larger randomized controlled trial. Another advantage to using ^{18}F -FDG PET/CT over other modalities is that full-body scanning can be used to obtain quantitative measures of articular ^{18}F -FDG. Therefore, ^{18}F -FDG PET/CT imaging offers an unprecedented ability to explore whether treatment-associated changes in vascular inflammation parallel changes in articular inflammation.

Although ^{18}F -FDG PET CT is sensitive to measuring the change in arterial inflammation, it is not a feasible tool for assessing and tracking ASCVD risk in the routine clinical care of patients with RA owing to its cost and radiation exposure. Ultimately, biomarker surrogates that indicate the extent that RA immunomodulation is effective in reducing arterial inflammation will be needed. Although it seems likely that clinical assessments of RA disease activity, such as the number of swollen and tender joints and routinely obtained systemic inflammatory markers (eg, the erythrocyte sedimentation rate and circulating C-reactive protein [CRP]), will improve in parallel with arterial inflammation, this has yet to be established. A broader array of circulating biomarkers may be required to identify patients with RA who would have actionable levels of arterial inflammation and/or those whose treatment has reduced their arterial inflammation to an extent at which their ASCVD risk has decreased.

TARGET TRIAL DESIGN

Overview. The primary objective of the TARGET (Treatments Against RA and Effect on ^{18}F -FDG PET-CT) trial is to compare the ability of two common DMARD regimens to reduce ^{18}F -FDG uptake in the aorta and carotid arteries of patients with RA who

have had an inadequate clinical response to MTX. The regimens tested will add either a TNF inhibitor (etanercept or adalimumab at standard doses) to background MTX or add SSZ and HCQ to background MTX (ie, "triple therapy"). Although neither subjects nor site investigators will be blinded to treatment allocation, the joint count assessor at each site will be blinded. In addition, all those involved in central data interpretation (ie, the trial primary investigators, imaging readers, and data analysts) will be blinded to treatment allocation.

Study population. To enrich the study population for vascular disease, all patients will be at least 45 years old for men and 50 years old for women. Subjects will fulfill American College of Rheumatology/European League against Rheumatism criteria for RA and will be deemed MTX-inadequate responders (ie, Disease Activity Score 28 [DAS28] > 3.2) by their treating rheumatologist. Individuals must be on MTX for 8 weeks or longer at a dose of at least 15 mg weekly (or on at least 7.5 mg for ≥ 8 weeks with a documented intolerance) and on a stable dose for the previous 4 weeks prior to screening. Additional details on the inclusion criteria are provided in Table 1.

Primary exclusion criteria include prior recent use of biologic DMARDs or Janus kinase inhibitors in the past 6 months or ever use of rituximab; consideration as a prior etanercept or adalimumab failure by their primary rheumatologist; use of nonbiologic DMARDs other than MTX or HCQ for 2 months prior to screening; current use or use within the past 12 months of a high-intensity statin lipid-lowering drug or PCSK9 inhibitor; and prior patient-reported, physician-diagnosed cardiovascular disease events (eg, MI, angina, stroke, uncompensated or severe heart failure, or vascular procedures). Background prednisone of more than 10 mg per day is not allowed, and limited changes over the course of the trial were permitted. Because of the use of ^{18}F -FDG PET/CT to

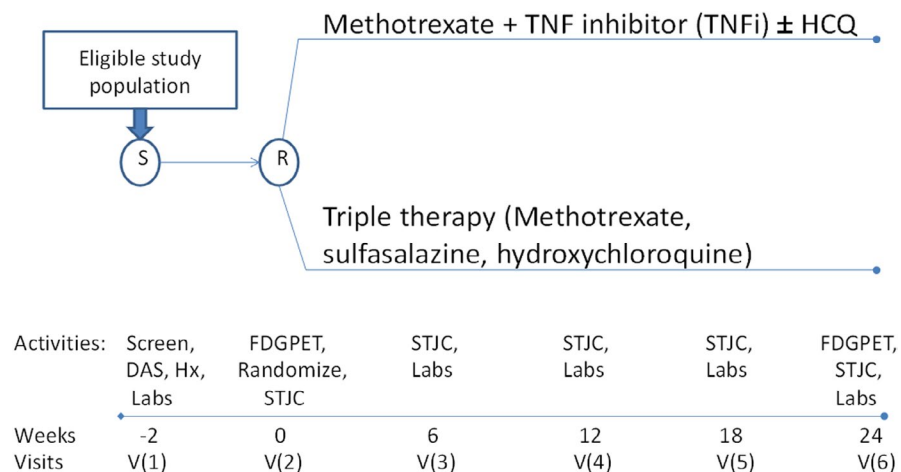


Figure 1. The diagram summarizes the overall study design. The time point at which study visit is performed is indicated above the visit number. Laboratories include blood draws and biomarker measurement. DAS, disease activity score; FDGPET, 18-fluorodeoxyglucose positron emission tomography/computed tomography scan; HCQ, hydroxychloroquine; Hx, health history; labs, laboratories; R, randomization; S, initial screening; STJC, swollen/tender joint count; TNFi, tumor necrosis factor inhibitor; V, visit.

assess study outcomes, intra-articular corticosteroid injections will not be allowed within the 4 weeks prior to the baseline scan. Those with two or more high radiation-dose scans in the past year will be excluded. Additional exclusion criteria are described in Table 1.

Summary of study design. The overall design of the TARGET trial is described in Figure 1. Patients who consent and meet eligibility criteria will undergo ^{18}F -FDG PET/CT scanning. If no significant incidental findings are identified that preclude randomization, the subject will be randomized. Randomization will be performed centrally using a permuted block design stratified by use at baseline of statins, oral steroids, and HCQ.

Visits will be performed at 6-week intervals. Blood will be drawn at each visit for safety monitoring. Fasting blood specimens will be drawn at Visits 2, 3, 5, and 6 (Figure 1) for the research biospecimen repository. At each visit, medication adherence will be assessed using self-report and pill counts, and the blinded metrologist will perform a joint count. Each of 44 joints will be examined for tenderness (subject “yes” or “no”) and swelling (metrologist “yes” or “no”). Repeat ^{18}F -FDG PET-CT scanning will be performed at 24 weeks.

Sites will be responsible for reporting adverse events to the coordinating center via the electronic data capture system. The adverse event report will include a description of the event and the site investigator’s assessment of expectedness, relatedness, and other relevant information. Anticipated adverse events are specified in the package inserts of the study drugs.

The National Institutes of Health will assign a Data Safety and Monitoring Board (DSMB) for this study.

Treatment algorithm. The treatment algorithm is summarized in Figure 2. Subjects in the TNFi arm will receive either 50 mg etanercept subcutaneously weekly or 40 mg adalimumab subcutaneously every other week. Subjects will continue the same dose of their background MTX and, if applicable, HCQ. If the subject’s Clinical Disease Activity Index (CDAI) score is greater than 10 at 18 weeks, treatment will be switched to the alternate TNFi.

Subjects assigned to the triple therapy arm will begin 500 mg SSZ twice daily and 200 mg HCQ twice daily, not to exceed 6.5mg/kg HCQ. Subjects will continue the same dose of their concomitant MTX. At 6 weeks, SSZ will be increased to 1 g twice daily for all subjects. If the subject’s CDAI score is greater than 10 at 18 weeks, MTX will be switched to 20 mg leflunomide daily.

All study medications will be provided to participating subjects, excluding MTX because subjects are already taking MTX prior to enrollment.

Study treatment may be discontinued for any subject who experiences any of the following: a repeat extreme laboratory value, malignancy other than basal or squamous cell carcinoma, repeated subject noncompliance or loss to follow-up, withdrawal of consent, investigator or DSMB belief that it is in the subject’s best interest, or termination of the study. Extreme laboratory

values include estimated glomerular filtration rate less than 30 ml/min/1.72m², white blood cell count less than 3000 n/μl, platelet count less than 50000 n/μl, hematocrit less than 27%, aspartate aminotransferase greater than 120 mg/dl, or alanine aminotransferase greater than 168 mg/dl.

If study treatment is discontinued (eg, for an adverse event) or if the subject withdraws from treatment, the subject will be asked to return for the follow-up FDG PET/CT scan before 24 weeks if he/she has received at least 8 weeks of the randomized treatment prior to withdrawal and if there is no safety issue precluding the scan.

^{18}F -FDG PET/CT imaging. Subjects will undergo ^{18}F -FDG PET/CT scans at baseline and at 24 weeks at their local site. All scans will be assessed centrally by trained nuclear cardiologists. The ^{18}F -FDG PET/CT scans will be assessed for arterial ^{18}F -FDG uptake in the ascending aorta and bilateral carotid arteries using a standardized protocol (46) Subjects will fast overnight before each scan and will abstain from carbohydrates and dairy at their last meal. A serum glucose will be obtained immediately prior to the scan and will be less than 150 mg/dl to proceed. At 90 minutes after ^{18}F -FDG injection, the CT attenuation-correction scan is performed, followed by the PET scan of the chest, neck, and joints. Sites will perform a safety read within 72 hours to identify any incidental findings.

Outcomes. The primary outcome is change in vascular ^{18}F -FDG uptake from baseline to follow-up while on the study drug using methods reported previously (38,47–50). Image analysis will be performed by an experienced reader with paired attenuation image sets of the vessels of interest evaluated side by side with scrambled time points and blinding to treatment (51). The target tissues will be matched such that the same locations are measured for both time points. Thereafter, regions of interest will be drawn around the target vessel (in axial orientation) to provide maximum standardized uptake values (SUVs) for each region of interest. The SUV is the decay-corrected tissue concentration of ^{18}F -FDG (in kBq/ml) divided by the injected dose per body weight (kBq/g). Drawing of regions of interest will be repeated along the length of the vessel (approximately every 3.5 mm along the long axis of the vessel) to provide a stack that comprises the whole vessel (51). Background (using the superior vena cava blood pool) corrected maximum SUVs will be averaged to provide a whole vessel target-to-background ratio (TBR). Absence of correction for partial volume effect has not been a limitation in the assessment of larger vessels, such as the carotids and the aorta, as shown by published validation studies (52).

The primary PET/CT parameter used to evaluate vascular inflammation will be the mean of the maximum TBR of the most diseased segment (MDS) of the index vessel (index vessel MDS $\text{TBR}_{\text{meanmax}}$), based on work showing that this endpoint provides the most sensitive measure of treatment effect (49,51). The index

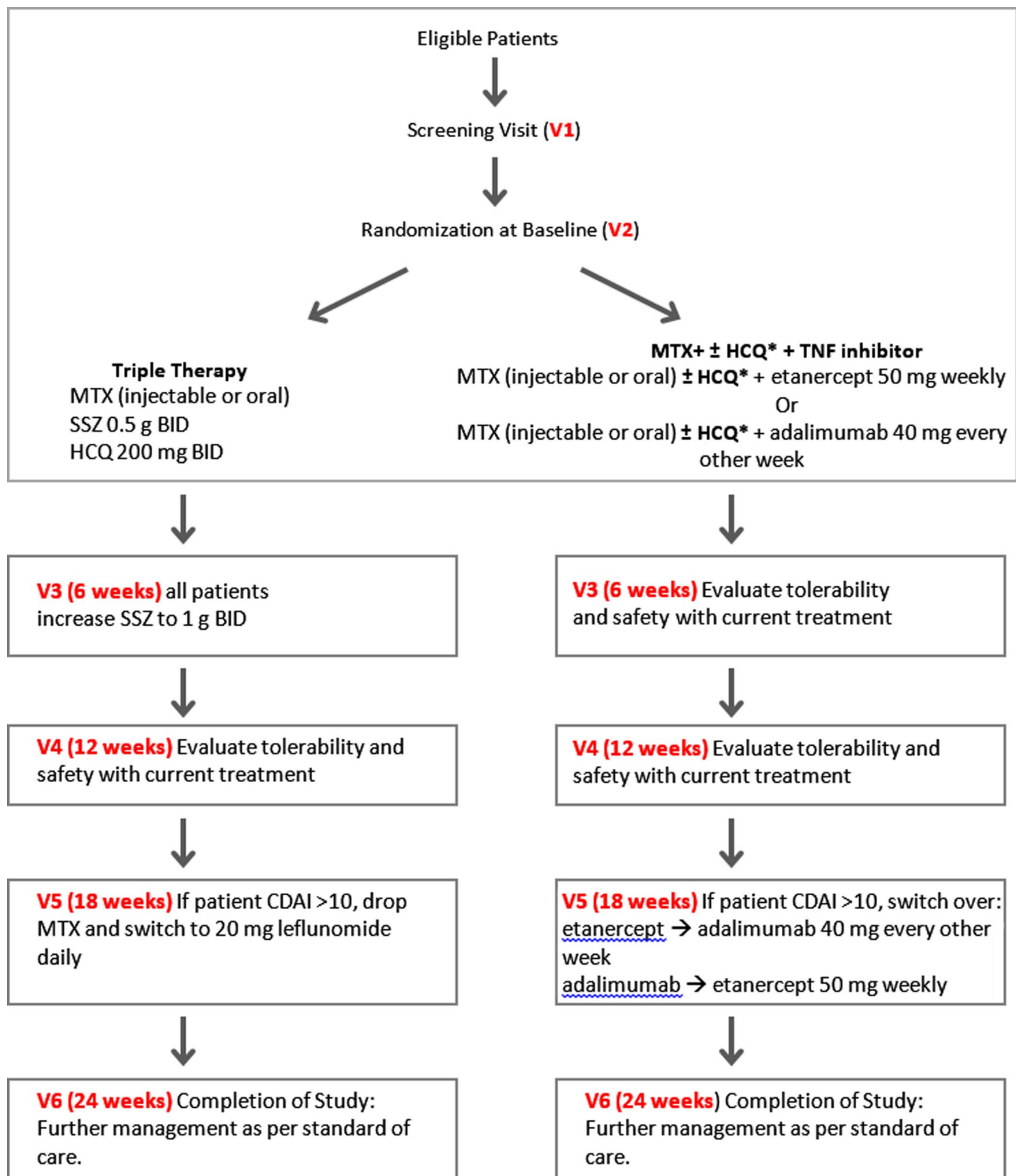


Figure 2. Treatment algorithm for participants in the TARGET (Treatments Against RA and Effect on 18-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography) trial. *Subjects entering the study on concomitant hydroxychloroquine (HCQ) and assigned to the tumor necrosis factor inhibitor (TNFi) arm will continue to take HCQ at its original dose. **Subjects entering the study on concomitant HCQ at <200 mg twice daily (BID) who are assigned to triple therapy will increase their HCQ dose to 200 mg BID provided that this new dose does not exceed 6.5 mg/kg. MTX, methotrexate; SSZ, sulfasalazine; V, visit.

vessel is the vessel (either aorta, left carotid, or right carotid) with the highest average $TBR_{meanmax}$ at baseline. The MDS is defined as the 1.5-cm segment within the artery that demonstrates the highest ^{18}F -FDG activity. The MDS $TBR_{meanmax}$ is calculated as a mean of maximum TBR values derived from three contiguous axial segments. The following secondary vascular imaging endpoints will also be examined in exploratory analyses: 1) the $TBR_{meanmax}$ across the entire index vessel, 2) carotid MDS $TBR_{meanmax}$, 3) aortic MDS $TBR_{meanmax}$, 4) $TBR_{meanmax}$ across the entire carotid vessel (right and left averaged), and 5) $TBR_{meanmax}$ across the entire aorta. These provide information complementary to the primary outcome.

Other secondary endpoints include changes in biomarker levels, specifically from the multi-biomarker disease activity (MBDA) test (VECTRA; Crescendo Bioscience Inc., South San Francisco, CA) [Crescendo/Myriad CA] and its individual components, change in articular ^{18}F -FDG uptake, and change in DAS28. The Vectra score is a validated correlate of RA disease activity (53). It is comprised of 12 biomarkers that were selected and weighted for their association with RA disease activity and which have also been associated with cardiovascular disease risk (54). The VECTRA score will be used to categorize patients as having remission, low, moderate, or high disease activity according to the manufacturer's algorithm (53,55). We will also analyze each of the 12 components of the Vectra test alone as continuous variables and in various combinations to determine whether a different algorithm of the Vectra components may be a better correlate of vascular inflammation. Using a recently developed method to measure articular ^{18}F -FDG uptake, we will calculate the change in the following measures from baseline to 24-week follow-up: number of ^{18}F -FDG-positive joints of 28 joints (primary articular endpoint; PET-28), the number of ^{18}F -FDG-positive joints of 68 joints, the mean maximum SUV of 28 joints, and the mean maximum SUV of 68 joints. Change in DAS28 will be calculated using the blinded metrologist assessments of total joint count and swollen joint count, patient-reported global arthritis activity, and CRP levels.

Sample size. From previous data in patients with RA, a baseline MDS maximum TBR of 2.51 (SD = 0.33) and a 0.46 reduction after 8 weeks of a TNFi (10) were observed. Based on these, we originally proposed a recruitment target of 200 patients. Later, after discussions with the DSMB, the sample size was re-estimated using blinded data from the first 45 TARGET trial enrollees. The re-estimated SD was 0.29, allowing for a smaller effective sample size of 126 (150 randomized minus 24 potential dropouts [~15%]). Accordingly, the trial has 99% power to detect an absolute difference of 0.17 TBR units between the two arms. This difference corresponds with the effect observed in the prior study by Maki-Petaja et al (10) and with the difference in effect observed between low- and high-dose statins (46), a contrast with known clinical significance (56). Power will be sufficient for the per-protocol secondary analysis, including only the approximately 75% of

subjects who remain adherent to study treatment. This prespecified secondary analysis will include an estimated 56 subjects per arm and will have 99% power to detect a difference of 0.17 in MDS $TBR_{meanmax}$. Because this is a prespecified secondary analysis, *P* values will not be adjusted.

Statistical analysis plan. *Analyses 1 and 2.* Overall, Analysis 1 will primarily determine the effects on vascular inflammation of TNFi and MTX versus triple therapy. A secondary analysis will explore whether these effects are mediated by changes in disease activity. The first part of this analysis will use an analysis of covariance (ANCOVA) model estimating the final index vessel MDS $TBR_{meanmax}$ as a function of the baseline index vessel MDS $TBR_{meanmax}$, treatment group, and randomization strata. A *P* value threshold of 0.05 for a two-sided test will be used to determine statistical significance. The primary analysis will only include participants with imaging data at baseline and follow-up.

The secondary analyses will follow an approach similar to that used in the recent mediation analyses of the Canakinumab Antiinflammatory Thrombosis Outcome Study trial anemia data, examining whether the magnitude of the treatment response achieved by individuals is related to their vascular inflammation response (57). This analysis will divide the participants into four groups according to randomized treatment assignment and whether they achieved low disease activity or remission versus remaining at moderate to high disease activity as defined by the DAS28 at 18 weeks. The four groups will be defined as 1) triple therapy remaining in moderate/high disease activity (reference group), 2) triple therapy achieving low disease activity/remission, 3) TNFi and MTX remaining in moderate/high disease activity, and 4) TNFi and MTX achieving low disease activity/remission. We chose to examine the DAS28 at 18 weeks as the mediator to capture the effects prior to any medication changes and prior to the assessment of the outcome. We will use an ANCOVA model estimating the final index vessel MDS $TBR_{meanmax}$ as a function of the baseline index vessel MDS $TBR_{meanmax}$, randomization strata, the four groups (combining information on treatment assignment and treatment response described above), a term for length of time between baseline and follow-up PET/CT scan, and the following potential confounders as measured at baseline: age, sex, disease duration, smoking status, serologic status, and body mass index (BMI).

We will also use formal causal mediation analysis to control for baseline characteristics that might independently influence treatment response and to control for the effect of treatment (58). We will perform analyses using low disease activity or remission at 18 weeks and DAS28 at 18 weeks as mediators. We will include a term for length of time between baseline and follow-up PET/CT scan and control for the following potential confounders, as measured at baseline: age, sex, disease duration, smoking status, serologic status, prednisone use, statin use, and BMI.

Additional secondary analyses will repeat the main analysis of the primary outcome in the subgroup adherent to study treatment (threshold of >80% of treatment days covered). Other important subgroups will also be analyzed within assigned treatment groups using an interaction term; these include achievement of low disease activity or remission, serologic status, at least one cardiovascular risk factor (eg, hypertension, diabetes, tobacco use, or hyperlipidemia), and statin initiator exclusion. An additional secondary analysis will compare the primary vascular inflammation outcome (MDS TBR_{meanmax}) change between adalimumab and etanercept users. Because of the coronavirus disease 2019 (COVID-19) pandemic, some sites were unable to perform research PET/CT scans according to the timeline specified in our protocol. We amended the protocol in April 2020 and allowed participants to be scanned after up to 36 weeks of treatment. Given potential differences in time between baseline and follow-up PET/CT scans because of the COVID-19 pandemic, we will also perform additional analyses controlling for length of time between PET/CT scans. These subgroup analyses will be underpowered and exploratory; we will report nominal *P* values.

For our next set of analyses (Analysis 2), we will use a similar overall methodology as described above for the primary outcome of MDS TBR_{meanmax} but the outcome of interest will be joint inflammation as measured by articular ^{18}F -FDG PET/CT (PET-28).

Analysis 3. Although prior work has explored the association between Vectra score and DAS28, less is known about the association between Vectra score and measures of vascular inflammation and articular inflammation. Our third set of analyses are exploratory and will pool data across both treatment arms to obtain additional insights into potential associations between biomarkers and vascular and articular inflammation.

First, we will compare baseline measurements of the Vectra score to the baseline vascular inflammation assessment (MDS TBR_{meanmax}) using linear regression. Second, baseline to 6-month change in Vectra score will be compared with the 6-month change in MDS TBR_{meanmax} . Finally, the 18-week change in Vectra score will be compared with the 6-month change in MDS TBR_{meanmax} , including the same set of covariates. All analyses will adjust for treatment arm, length of time between baseline and follow-up PET/CT scan, age, sex, disease duration, smoking status, serologic status, baseline disease activity (DAS28), prednisone use, statin use, and BMI. We will repeat these analyses using the measures of articular inflammation (PET-28) as the outcomes instead of vascular inflammation.

Analysis 4. In exploratory analyses, we will compare the change in vascular inflammation (MDS TBR_{meanmax}) with the change in articular inflammation (PET-28). This will be examined across both treatment arms and then individually by treatment arm. The change in articular inflammation (PET-28) will be considered the independent variable, and the change in MDS TBR_{meanmax} will be the dependent variable. These analyses will use linear regression and will include the same set of covariates

as noted in Analysis 3. We will also examine subgroups, including by prednisone use (yes/no), statin use (yes/no), treatment arm (triple therapy/TNF antagonist), serologic status, and sex.

CONCLUSION

The TARGET trial will be the first randomized clinical trial to test the comparative efficacy of any RA immunomodulators on inflammation reduction in a nonarticular target tissue. Because ASCVD is a key source of morbidity and mortality in RA and observational data provide only a limited and discrepant suggestion that RA DMARDs reduce ASCVD risk, the findings of the TARGET trial have the potential to influence the way that DMARDs are prescribed for people with RA. Moreover, the trial has the potential to establish surrogate circulating biomarkers that will indicate those with unrecognized and actionable arterial inflammation.

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AUTHOR CONTRIBUTIONS

All authors drafted the article, revised it critically for important intellectual content, approved the final version to be published, and take responsibility for the integrity of the data and the accuracy of the data analysis.

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