Effects of **A**batacept on **M**yocarditis **i**n **R**heumatoid **A**rthritis

(AMiRA)

Clinical Trial Protocol

IRB Protocol #: AAAS2235

BMS protocol #IM101-782

NCT03619876

Investigators:

Laura Geraldino-Pardilla, MD, MSc (PI). T- (212) 305-4308

Joan M Bathon, MD (co-I). T- (212) 305-6327

Robert Winchester, MD (co-I). T- (212) 305-8250

Sabahat Bokhari, MD (co-I). T- (212) 305-5996

Rachel Broderick, MS (Research coordinator). T- (212) 342-2713

Christopher Depender (Research Coordinator). T- (212)305-4114

Trial Registration Number: NCT03619876

Version 4.3: DEC 06 2019

STUDY SYNOPSIS

Animal data showing decreased myocardial inflammation, damage, and mortality, and improved cardiac function with CD40L/B7-1 and CTLA4 blockage, coupled with our preliminary findings of lower myocardial inflammation in RA patients on abatacept vs other DMARDs, suggest that abatacept treatment has potential myocardial benefits. In RA patients, the proportion of peripheral T cell subsets significantly differs from normal controls and include differentiation to memory effector subsets, acquisition of NK receptors, exhaustion markers, and enhanced inflammatory cytokine expression. Importantly, T cell lymphocytic infiltration described in autoimmune myocarditis resulting as a complication of CTLA4 immune checkpoint inhibition, suggests a role for T cell subsets in the pathogenesis of myocarditis in RA with potential differences depending on mechanism of action of the DMARD in use.

This study aims to evaluate the effects of abatacept, a CTLA4-Ig fusion protein that binds CD80/86 (B7-1/B7-2), on subclinical myocarditis in rheumatoid arthritis (RA) through its effect on T cell subpopulations. RA patients without clinical CVD, biologic naïve, and with inadequate response to methotrexate (MTX), will undergo cardiac FDG PET/CT imaging to assess myocardial inflammation. Studies that investigate the impact of treatment on subclinical myocarditis in RA, a possible contributor to heart failure, while exploring potential underlying mechanisms (i.e. different T cell subpopulations), are needed for a better understanding of their relevance in the pathogenesis of heart failure in RA and survival improvement in these patients with excess risk for cardiovascular death. If our hypothesis is confirmed and treatment with abatacept decreases and/or suppresses or prevents myocardial inflammation in RA, this will have multidisciplinary implications that could lead to changes in the current management of RA patients at high risk for cardiovascular events. Similarly, identification of T cell subpopulations in RA patients with myocardial FDG uptake will shed light into the underlying cellular mechanisms of myocardial injury and serve to guide the use of therapies that prevent their pathogenicity.

The objectives of this study are to compare the change in myocardial FDG uptake in RA patients treated with abatacept vs adalimumab, and identify T cell subpopulations associated with myocardial FDG uptake in each treatment arm. RA patients will be randomized in an unblinded, 1:1 ratio to treatment with abatacept vs adalimumab. A cardiac FDG PET/CT will be performed at baseline and 16 weeks post-biologic treatment. T cell subpopulations associated with myocardial

FDG uptake will be evaluated at both points in time with their transcriptional phenotype outlined by RNA seq.

Inclusion/Exclusion Criteria.

Inclusion Criteria: Patients age ≥ 18 years, fulfilling the American College of Rheumatology 2010 classification criteria for RA (60), with inadequate response to MTX, and not on recent biologic treatment, will be recruited from the rheumatology clinics of Columbia University Medical Center and by referral from local rheumatologists.

Exclusion criteria: 1) Prior biologic use in the past 3 months, or rituximab use in the past 6 months; 2) any prior self-reported physician diagnosed CV event (myocardial infarction; angina; stroke or Transient Ischemic Attack (TIA); heart failure; prior CV procedure (i.e., coronary artery bypass graft, angioplasty, valve replacement, pacemaker); 3) Active or on-treatment cancer within 5 years of baseline visit or prior use of immune checkpoint inhibitors; 4) Known pregnancy, HIV, hepatitis B, hepatitis C, active (or untreated latent) tuberculosis; 5) Prisoners or subjects who are compulsory detained

Statistical Analysis. Summary statistics for outcomes and predictor variables will be examined; with comparisons made using student t-test and Wilcoxon rank-sum test for normally and non-normally distributed continuous variables, respectively. Counts and percentages will be calculated for categorical variables and compared using the chi-square or Fisher's exact test, as appropriate. In Aim 1, using generalized estimating equations (GEE), models will be constructed to explore the association between treatment arms (abatacept vs adalimumab) and change in myocardial FDG uptake treated as a continuous variable (measured by SUV units) to take into account patients' baseline uptake values. Similarly, linear mixed effects models will be used to define the association between T cell subpopulations and myocardial FDG uptake per treatment arm strata. Given the small sample size of the study, we will not have the power to adjust for multiple variables with the exception of anti-CCP seropositivity, but the two treatment arms will be matched for age, sex, and disease activity, and the contribution of RA-disease characteristics and traditional CVD risk factors to the variability of myocardial FDG uptake will be ascertained in the univariate analysis. All statistical calculations will be performed using SAS 9.4. In all tests, a 2-tailed α of 1.5 will define statistical significance.

Accrual goal and rate. Patient enrollment will take place over a period of 2 years from initiation of the study. With aims of enrolling 10 patients per year, the enrollment rate is estimated as 1 patient per month.

CONSORT Flow Diagram

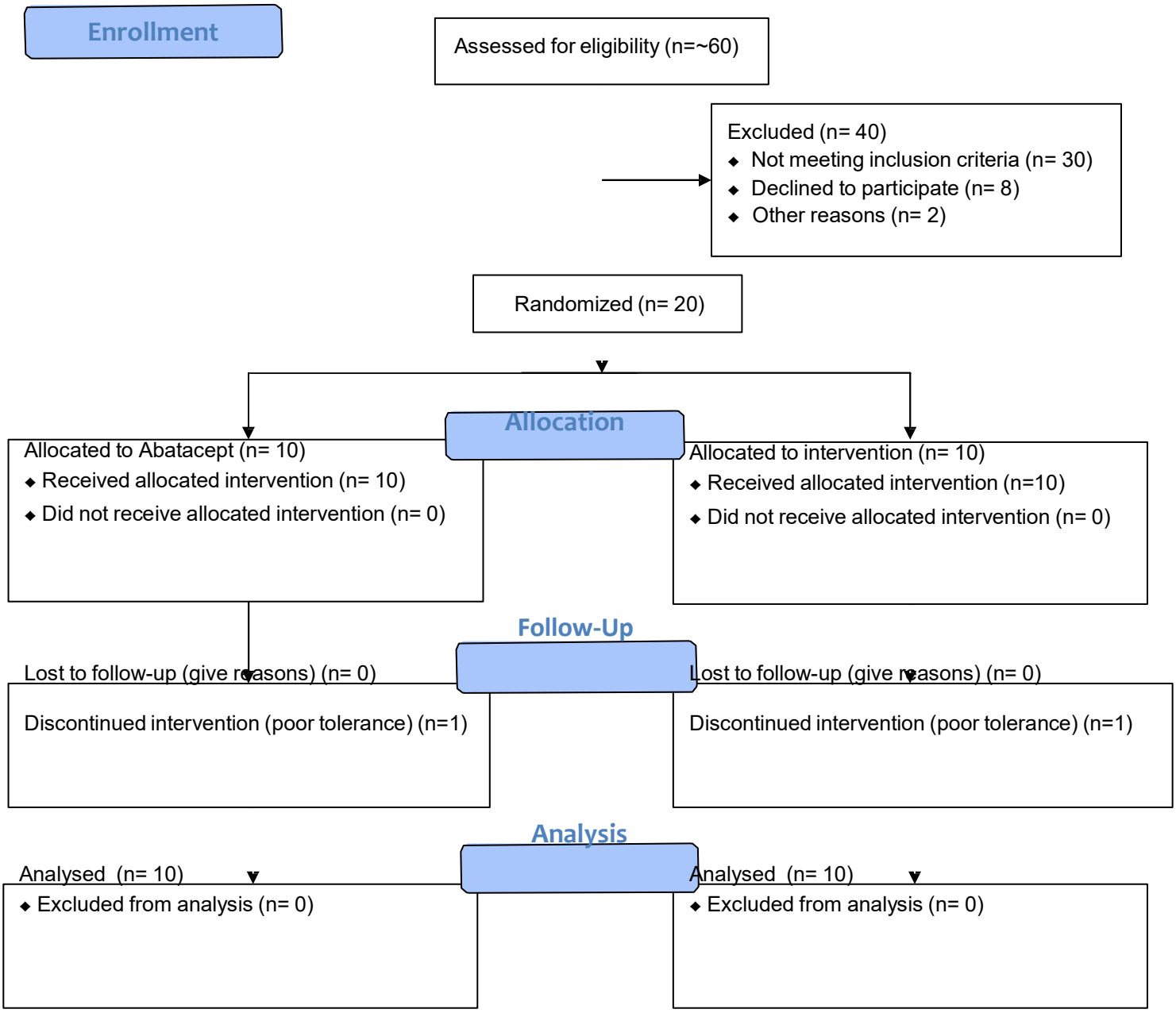


TABLE OF CONTENTS

1.BACKGROUND

1.1.Background information.

1.1.1 Overview. Rheumatoid arthritis (RA) is a systemic inflammatory disease that affects ~1% of the population. Regardless of the novel therapies developed in the last decades, studies report an increased standard mortality ratio as high as 3.0 when compared with the general population (1). Cardiovascular disease (CVD) is the leading cause of mortality in RA subjects in whom the average lifespan is reduced by 8-15 years compared to matched controls (1-5). RA patients are at increased risk for developing heart failure (2,3) and inflammatory myocarditis potentially contributes to this excess risk (9-11). Although subclinical myocarditis remains poorly characterized to date in RA, costimulatory molecules such as CD80/86 (B7s) and CD40 are known to play a pivotal role for cytokine production and antigen-specific T cell activation in viral myocarditis, and in murine models, blocking CD40L/B7-1 and CTLA4 significantly decreases myocardial inflammation, damage, and mortality (15-18). In addition, the recent increase in the use of immune checkpoint inhibitors for the treatment of numerous cancers, has raised awareness of the occurrence of fulminant autoimmune lymphocytic myocarditis as a complication of these drugs including anti-CTLA4 due to a presumed uncontrolled immune response resulting in T-cell mediated myocardial injury (19,20). Interestingly, our pilot data showed lower myocardial FDG uptake in RA patients on the a CTLA4-Ig fusion protein abatacept compared with other DMARDs. These data raise the possibility of immunotherapy for the treatment of myocarditis in RA, suggesting a role for T cell infiltration in its pathogenesis, and a particular benefit for treatment with abatacept vs non-abatacept biologic DMARDs.

1.1.2. Myocarditis in Rheumatoid Arthritis. Autopsy studies of RA hearts from the 1960s suggest that myocarditis may occur in as many as 16% of patients (9-11). There is scarce data on myocarditis in contemporaneous RA history which is itself restricted to data in patients with known history of CVD (12). Direct examination of the RA myocardium has been limited due to reliance on endomyocardial biopsy (EMB), which is invasive and has poor sensitivity to detect myocarditis due to its heterogeneous and patchy nature (13-14). This, coupled with its invasiveness, expense and risk of complications, has limited investigations of subclinical myocarditis in RA. However, recent advances in myocardial imaging techniques have shown great promise in detecting myocardial inflammation. Cardiac MRI studies in RA using late

gadolinium enhancement (LGE) suggest an increased prevalence of cardiac LGE in RA compared with controls (30-32). Yet, the detection of myocardial LGE is performed by contrast to the background enhancement, rendering the visualization of diffuse LGE difficult to assess (30, 32-33), and makes it a suboptimal tool for diagnosing myocarditis in RA due to its limitation in distinguishing inflammation, from fibrosis, edema, and necrosis. A more recently used MRI technique: T2-weighted imaging allows for better specificity in regards to inflammation but seems to be less sensitive and currently data on RA patients without CVD using this imaging modality remains scarce (31). While there hadn't been any data in RA using cardiac FDG-PET-CT prior to our recent study, this technique is proving to be a valuable diagnostic tool in other inflammatory disorders such as cardiac sarcoidosis and viral myocarditis where it can detect myocardial inflammation with greater sensitivity than EMB (34-36). FDG is a glucose analog taken up by metabolically active cells including macrophages in regions of inflammation (37-40) and cellular FDG uptake reflects the rate of tissue glycolysis (high in inflamed tissues), thus resulting in avid uptake (41-43). In experimental models of myocarditis and atherosclerosis, FDG uptake was confirmed to correlate with histological demonstration of infiltrating macrophages and/or CD3+ T cells (44-45). Because cardiomyocytes are also metabolically active but have different glucose receptors compared to inflammatory cells, cardiomyocyte glucose uptake can be suppressed and inflammatory cell uptake isolated and detected (41-43). To date, data on FDG myocardial uptake response to DMARDs in RA is lacking.

1.1.3. Cytotoxic T lymphocyte–associated antigen 4 (CTLA4), CTLA4-Ig, and Myocarditis. CTLA4 is an essential costimulatory molecule involved in the downregulation of T-cell activation. CTLA4 is expressed on the activated T-cell surface and inhibits responses by competing with CD28 for CD80/86 (B7s) binding, effectively preventing CD80/86 (B7s)-CD28 interactions. CTLA4-Ig is a fusion protein that combines the CTLA4 extracellular domain and has high affinity for ligand CD80/86 (B7-1/B7-2), hence blocking T-cell costimulation. This mechanism is of particular interest in viral myocarditis as T-cell mediated cellular immunity is considered the most important histopathological feature in this condition (46). In experimental autoimmune myocarditis models, CTLA4-Ig has been shown to decrease or inhibit myocardial inflammation, virus replication, and mouse mortality, as well as improve cardiac function, presumably by influencing the balance of TH1 to TH2 T cells (15-18). Furthermore, the development of myocarditis in patients treated with immune checkpoint inhibitors is a recently recognized complication. Early, progressive and refractory cardiac electrical instability, and myocarditis with a robust presence of

T cell and macrophage infiltrates have been described in 0.27% of patients treated with a combination of anti-CTLA4 and anti-PD-1 therapies, and can result in a potentially fatal, T cell driven myocardial injury (19). Of note, the histopathology of these cases show a patchy lymphocytic infiltrate within the myocardium that also involves the cardiac sinus and atrio-ventricular nodes, with lymphocytic destruction of isolated myocytes, and predominantly infiltrating CD3+ (rich in both CD4+ and CD8+ T cells, and negative for CD20) or CD68+ cells (19). Overall, the deletion of CTLA4 and PD-1 axes can cause autoimmune myocarditis and dilated cardiomyopathy, supporting that these molecules play a role in the prevention of T-cell mediated autoimmune myocarditis (19,20).

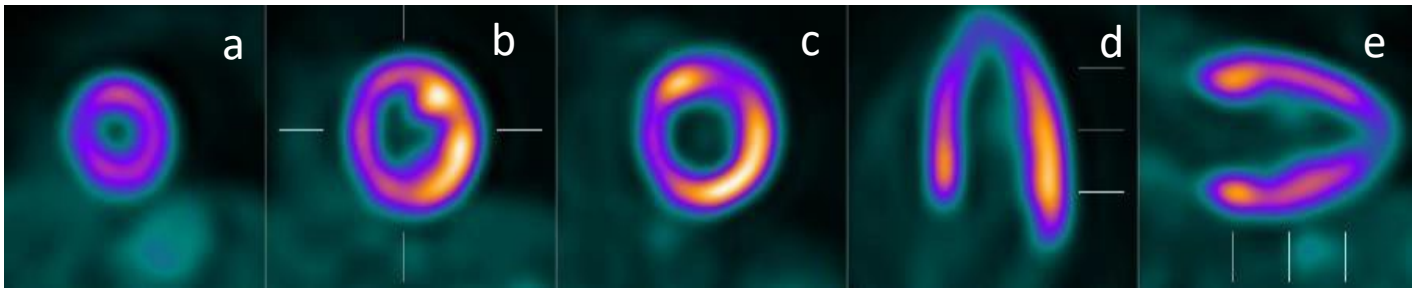
1.1.4. T cells in RA. In an adaptive immune response, activation of antigen-specific T cells, often denoted by HLA-DR expression (47), is followed by clonal expansion and progressive T cell differentiation along defined maturation pathways involving transcriptional regulation of various molecules governing recognition and response. Their patterns of acquisition and loss define functionally different T cell subsets involved in protective memory and immune effector functions (48). Their differentiation from the naïve state to a memory-effector phenotype is denoted by extinction of the expression of CD28 (CD4+CD28null T cells) and a shift from the CD45RA isoform to CD45RO, with pro-inflammatory and autoreactive potential (49-53). Activated, expanded, and differentiated memory effector T cells are widely considered to be an intrinsic feature of RA (54,55). Furthermore, in some RA patients, expression of natural killer (NK) cell receptors (CD56 and CD57) is also noted (56-58), and is of particular interest as it enables the CD4+CD28null T cells to be triggered by danger signal ligands expressed by stressed or injured cells (59), providing a new second stimulatory signal that along with the T cell receptor engagement result in cell activation in a site of injury or inflammation. However, the role of different T cell subsets in the pathogenesis of myocarditis in RA remains unclear.

2.RATIONALE.

2.1. ***Preliminary Data.*** In a single RHeumatoid arthritis studY of THe Myocardium (RHYTHM study), a total of 119 RA patients without clinical CVD underwent cardiac FDG-PET/CT, with myocardial inflammation assessed qualitatively and quantitatively by visual inspection and by calculation of the standardized-uptake-value (SUV) units. Qualitative myocardial FDG uptake was observed in 39% of the patients (Figure 1). By quantitative assessment, the median

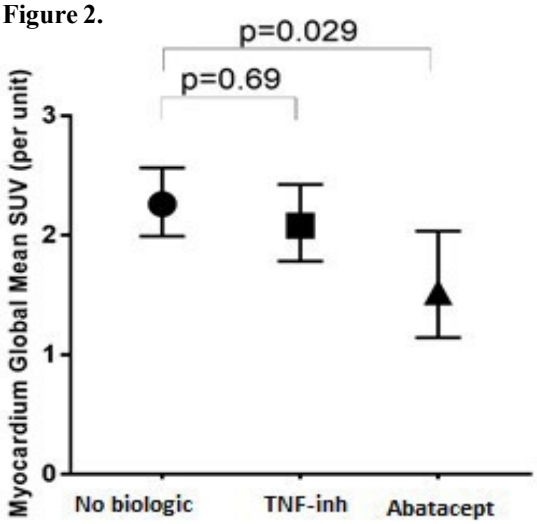
myocardial SUV mean was 1.9 (inter-quartile range [IQR]: 1.5-2.6), and the median SUV max was 3.0 (IQR: 2.1-4.5).

Figure 1. Example of diffuse Myocardial ^{18}F-FDG uptake Scan in an RA patient. Short Axis for a, b and c; horizontal axis for d; and vertical long axis for e.



As expected, RA patients with visually detected FDG uptake had higher SUVs by quantitative assessment than patients without visually detected FDG uptake (median SUV mean of 2.8 [IQR: 2.2-4.9] vs 1.6 [IQR: 1.4-1.9], p <0.0001), and median SUV max of 4.9 [IQR: 3.6-9.4] vs 2.3 [IQR 2,0-2.9], p <0.0001, respectively). Interestingly, when comparing the degree of myocardial FDG uptake in RA patients on abatacept vs no biologic agents; abatacept treatment (n=8) was associated with a lower SUV mean. This association remained statistically significant with an 18% lower myocardial SUV mean seen after adjusting for disease activity (Clinical Disease Activity Index less vs greater or equal to 10) (beta=-0.36) (Figure 2). No significant difference was seen between myocardial FDG uptake in those on non-biologic DMARDs vs TNF-inhibitors (p=0.69). In addition, higher CDAI and DAS-28 scores, non-Hispanic black race, and body mass index (BMI) were significantly associated with a higher SUV mean. A CDAI ≥10 (moderate/high disease activity) was associated with a 0.31 increase in SUV mean when compared to the group with CDAI in remission or low disease activity (CDAI<10) (beta=0.31, p=0.006). A similar relationship was observed with DAS-28. Non-Hispanic black RA patients had 22% higher SUV mean than non-Hispanic whites (beta=0.31, p=0.006). However, in adjusted analyses, only the CDAI≥10 remained significantly associated with a higher SUV mean.

Figure 2.



2.2. Scientific Premise. This study aims to evaluate the effects of abatacept, a CTLA4-Ig fusion protein that binds CD80/86 (B7-1/B7-2), on subclinical myocarditis in rheumatoid arthritis (RA) through its effect on T cell subpopulations. Animal data showing decreased myocardial inflammation, damage, and mortality, and improved cardiac function with CD40L/B7-1 and CTLA4 blockage, coupled with our preliminary findings of lower myocardial inflammation in RA patients on abatacept vs other DMARDs, suggest that abatacept treatment has potential myocardial benefits. In RA patients, the proportion of peripheral T cell subsets significantly differs from normal controls and include differentiation to memory effector subsets, acquisition of NK receptors, exhaustion markers, and enhanced inflammatory cytokine expression. Importantly, T cell lymphocytic infiltration described in autoimmune myocarditis resulting as a complication of CTLA4 immune checkpoint inhibition, suggests a role for T cell subsets in the pathogenesis of myocarditis in RA with potential differences depending on mechanism of action of the DMARD in use. Studies that investigate the impact of treatment on subclinical myocarditis in RA, a possible contributor to heart failure, while exploring potential underlying mechanisms (i.e. different T cell subpopulations), are needed for a better understanding of their relevance in the pathogenesis of heart failure in RA and survival improvement in these patients with excess risk for cardiovascular death. If our hypothesis is confirmed and treatment with abatacept decreases and/or suppresses or prevents myocardial inflammation in RA, this will have multidisciplinary implications that could lead to changes in the current management of RA patients at high risk for cardiovascular events. Similarly, identification of T cell subpopulations in

RA patients with myocardial FDG uptake will shed light into the underlying cellular mechanisms of myocardial injury and serve to guide the use of therapies that prevent their pathogenicity.

3.STUDY OBJECTIVES

The aim of this study is to evaluate the effects on myocarditis in rheumatoid arthritis (RA) of abatacept, a CTLA4-Ig fusion protein that binds CD80/86 (B7-1/B7-2).

3.1. Primary Objective. Compare the change in myocardial FDG uptake in RA patients treated with abatacept vs adalimumab. Rationale: In murine models, CD40L/B7-1 and CTLA4 blockade decreases myocardial inflammation, damage and mortality. Additionally, our pilot data suggests that lower myocardial FDG uptake in seen in RA patients on abatacept compared with other DMARDs. Approach: Using FDG PET cardiac imaging to identify myocardial inflammation at baseline and post-treatment, we will quantitatively compare the change in myocardial FDG uptake in biologic naïve RA patients without clinical CVD and with inadequate methotrexate response, following randomization to 16-week treatment with abatacept vs the TNF-inhibitor adalimumab. Conventional CVD risk factors and measures of RA disease activity and severity will be ascertained.

3.2. Secondary Objective. Identify T cell subpopulations associated with myocardial FDG uptake in RA patients treated with abatacept vs adalimumab. Rationale: Reflecting the adaptive immune response underlying RA, the proportion of peripheral T cell subsets in RA patients significantly differs from normal controls. These include differentiation to memory effector subsets, acquisition of NK receptors, exhaustion markers, and enhanced inflammatory cytokine expression. Furthermore, T cell lymphocytic infiltration has been described in autoimmune myocarditis resulting as a complication of CTLA4 immune checkpoint inhibition. These data suggest a role for T cell subsets in the pathogenesis of myocarditis in RA with potential differences depending on mechanism of action of the DMARD. Approach: We will test whether elevations in different T cell subsets are associated with myocardial FDG uptake in RA patients treated with abatacept vs adalimumab. Subsequently, the transcriptional phenotype of candidate subpopulations isolated by cell sorting will be comprehensively delineated by low cell input RNAseq to define the specific immunobiologic characteristics of these particular cellular subsets that are mechanistic candidates to mediate myocardial inflammation.

4.ETHICAL CONSIDERATIONS / INFORMED CONSENT

4.1. 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 subject's protection training.

4.2. Informed Consent. The principal investigator, co-investigators and research staff are responsible for obtaining informed consent before any subject may participate in the study. 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. The subject may withdraw consent to participate in the study at any time. The approved informed consent form will adhere to the ethical principles that have their origin in the Declaration of Helsinki.

4.3. Institutional Review Board. The study will be conducted under the monitoring of the local Institutional Review Board (IRB). The PI will be responsible for submitting the clinical trial protocol, the Informed Consent form, any advertisements, and all other relevant study related documents to the local IRB for approval. All unanticipated problems, as well as protocol deviations and violations that occur during the conduct of the trial will be reported by the PI to the IRB. Adverse events will be submitted regularly to the IRB. The IRB must approve all protocol changes prior to implementation unless emergency action is clinically indicated. The PI will be responsible for preparing annual reports to the IRB.

4.4. Training of Investigators and Staff for the Study Protocol. 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 staff members have been appropriately trained in the study protocol. Training and delegation of responsibility will be documented in a delegation of responsibility log.

5.STUDY DESIGN AND METHODOLOGY

5.1. Overview. The aim of this study is to prospectively evaluate the effects on myocarditis in RA of abatacept, a CTLA4-Ig fusion protein that binds CD80/86 (B7-1/B7-2), through its effect on T cell regulation. RA patients without clinical CVD, not on recent biologic DMARDs, and with inadequate response to methotrexate (MTX), will undergo cardiac FDG PET/CT imaging at baseline to assess myocardial inflammation. RA patients will be randomized in an unblinded, 1:1 ratio to treatment with abatacept vs adalimumab. A repeat cardiac FDG PET/CT will be performed16(±2) weeks post-biologic treatment. T cell subpopulations associated with myocardial FDG uptake will be evaluated at both points in time with their transcriptional phenotype outlined by RNAseq. Conventional CVD risk factors and measures of RA disease activity will be ascertained.

5.2. Study Population. Patients age $\geq$ 18 years, fulfilling the American College of Rheumatology 2010 classification criteria for RA (60), with inadequate response to MTX, and not on recent biologic DMARD treatment, will be recruited from the rheumatology clinics of Columbia University Medical Center and by referral from local rheumatologists. Exclusion criteria: 1) prior biologic use, other than rituximab in the past 3 months, or Rituximab use in the past 6 months; 2) any prior self-reported physician diagnosed CV event (myocardial infarction; angina; stroke or Transient Ischemic Attack (TIA); heart failure; prior CV procedure (i.e., coronary artery bypass graft, angioplasty, valve replacement, pacemaker); 3) active or on-treatment cancer within 5 years of baseline visit or prior use of immune checkpoint inhibitors; 4) Known pregnancy, HIV,

hepatitis B, hepatitis C, active (or

untreated latent) tuberculosis.

5.3. Study visits. Patients will be evaluated at baseline and 16 (±2) weeks post-biologic treatment, with an interim safety visit at week 8 (±2wks), and a final safety visit at week 20 (±2).

5.4. Imaging. Cardiac ^{18}F-FDG PET CT: Myocardial FDG uptake images will be assessed by FDG PET CT scanning. To suppress physiological uptake of FDG by cardiomyocytes, patient preparation will include a high fat non-carbohydrate diet the day before, and a 12-hour fast the evening prior to the scan (61-62). Dietary adherence will be interrogated the day of the scan. Imaging will be performed on a MCT 64 PET/CT scanner (Siemens Medical Solutions USA, INC., Knoxville, TN). A low dose CT transmission scan (120 kV, 25 mA) will be obtained for attenuation correction of PET data. Patients will be injected with 10 mCi ± 10% of ^{18}F-FDG intravenously. A list mode 3D PET scans will be acquired for 10 minutes following a 90-minute uptake period post-^{18}F-FDG injection. Non-gated attenuation-corrected images will be reconstructed yielding ~3 mm effective resolution. Corridor 4DM software will be used to visually assess myocardial ^{18}FDG uptake as well as semi-automatically quantify mean radiotracer uptake in the myocardium. Cardiac axes will be manually defined by marking the base on the vertical long axis, apex on the horizontal long axis, and the left ventricular cavity on the sagittal axis. FDG uptake will be assessed quantitatively as standardized uptake value (SUV), a measure of radiotracer uptake normalized for injected dose and patient weight, using polar maps and Corridor4DM v. 7.0 (Invia Medical Imaging Solutions, Ann Arbor, MI). Left ventricular segments will be defined by the segmentation nomenclature of the American Heart Association (63). Contours will be placed on the myocardial walls and automated SUV calculations for the mean SUV and the max SUV for each of the segments will be derived. The mean of the means (SUV mean) and the mean of the max SUVs (SUV max) will then be obtained.

5.5. Main Predictors. Treatment Arms: *Abatacept vs Adalimumab.* Treatment with abatacept will consist of weekly subcutaneous (SQ) injections at a dose of 125mg. Treatment with a adalimumab, as the TNF-inhibitor arm, will consist of every 2 weeks SQ injections at a dose of 40mg.

5.6. *Immunophenotyping of peripheral T cell subpopulations.* High-dimensional analysis of the mononuclear cells (MNLs) will be performed by multiparameter flow cytometry assessing the

per cell expression of over 50 differentially expressed molecules. These results will be used to delineate candidate predictor subsets that will be then used as variables to determine the presence of an association with myocardial uptake of FDG. Panels developed in Dr. Robert Winchester's laboratory will be used and will specifically focus on identifying the T reg subset, activated markers on T cells, exhaustion markers, and NK cells. This should result in a much more comprehensive immunologic characterization of the peripheral mononuclear cell alterations in RA and better enable identification of subsets related to myocardial inflammation. Distinctive populations not present in a reference set of RA cases with low or no myocardial FDG uptake will be identified by dimensional reduction analyses and corroborated by gating and biaxial plot analysis. The first step will be flow cytometry and will involve five panels, with each having a Zombie dye for live dead discrimination, CD3, CD4, CD8, CD45RA and CCR7 for identifying subsets across most panels, and on the T cell panels various combination of CD40L, CD31, HLA-DR, CD38, CD69, TIGIT, KLRG1, CD57, CD160, NKG2D, CD28, CD27, CD45RO, PD-1, CD95, CCR2, CCR4, CXCR3, CD39, CXCR5, CD62L, CCR6, CD25, CD161, IFN-γ, IL-17, TNF-α, FOX3P, RORγt, TBX21, GATA3, BCL6, HELIOS, IL-2. The NK/T activation panel also contains CD56, CD16, CD161, CD117, NKp44, NKG2A, CD158a, NKG2D, and CD94. Staining for each panel will be done in 30ul volumes on ~2 x106 MNLs. The cytometry will be done on a 6 laser LSRII. Samples will be run in batches, including samples from different clinical groups within each batch, with care taken to minimize inter-batch variation in staining. Every batch will include a cryopreserved reference normal MNL pool obtained by leukopheresis from 9 normal blood donors by the NY Blood Center, each validated for having a generally normal distribution of subsets and cryopreserved. This reference population will serve as a critical control for machine and technical variations in reagent performance, and will enable the identification of anomalous samples with low viability, enhanced aggregation, or other features suggesting sample processing or transport artifacts. Compensation will be done before each batch. In addition to the classic gating performed in FCS Express software, we will use multiple, complementary dimensional reduction approaches to analyze the multiparametric flow cytometry data (viSNE) as a way to obtain unbiased measures of the cell population alterations. First we will use the t-Distributed Stochastic Neighbor Embedding (t-SNE) algorithm viSNE (64) to visualize the multidimensional data and the various MNL subpopulations among circulating leukocytes (65). Here, the positions of cells on a 2D viSNE plot reflect their proximity in high-dimensional parameter space, including information from each of the dimensions. This unbiased alternative to traditional gating and biaxial subset representation preserves the geometry and nonlinearity of the data, delineates both abundant and rare populations without setting a priori expression criteria, and provides an interpretable data view

using color as a third dimensional to show the expression of particular differentiation and activation molecules. A generally similar implementation of viSNE is available in Cytobank, and both algorithms will be used in the delineation of distinctive MNL populations. In viSNE, the gene expression patterns of reference MNLs map into shapes that discriminate between immune subtypes, and the shape of these per cell patterns is altered in disease due to differences in gene expression resulting in patterns that are distinct from the referent control map. The area of the shape is proportional to the size of the population, and viSNE can identify populations consisting of 0.2% of the cell input (64). To compare the T cell subsets, we will first develop a referent viSNE map by overlying similarly grouped subpopulations and cells from the RA patients without myocardial FDG uptake. The similarity of each case subpopulation forming the referent distribution will be determined using the Shannon Diversity Index (SDI)(66), to quantify the similarity between the samples used to make the referent viSNE map. Samples used to make the referent map will be included if their SDI divergence between each pair is <0.05. We will then compare the immune cell populations in those with elevated myocardial FDG uptake by overlying their viSNE transformation to the referent viSNE map of those without FDG myocardial uptake, to define one or more multidimensional phenotypes that differ in each myocarditis patient from the referent. The relation of the population expression patterns to conventional immune subtypes can be corroborated by manual gating of a series of biaxial plots using the molecular markers distinguished in the viSNE plot. The comprehensively defined novel and conventional populations will be designated and their quantitative distribution across the subjects will be tabulated for analysis as predictor variables for Aim 2. As a second alternative and complementary dimensional reduction approach, we will utilize the CITRUS (cluster identification, characterization, and regression) (67) approach implemented through Cytobank (www.premium.cytobank.org). In this method, a modification of SPADE, cells are hierarchically clustered into nodes based on their multidimensional phenotype, and the occupancy of different nodes can be compared quantitatively across individual patients and patient groups. This allows the identification of unique cell populations based on multidimensional phenotypes and then assess for subpopulations that are increased or decreased in groups of patients of interest. Once an altered node is identified, the expression of markers can be assessed to determine defining features of cells in that node. An important feature of CITRUS is that the discovery of statistically significant stratifying MNL subpopulations in these datasets is facilitated by its structure that compares sets of cases across multiple discriminating characteristics (i.e., FDG myocardial uptake positive versus FDG uptake negative). The features of the population(s) defined in viSNE will then be compared with those identified in CITRUS to provide a common set of weighted features. Differences in the putative

same population between the two methods can be resolved by back gating and analysis of the differences by conventional biaxial displays to characterize the phenotypic elements responsible for divergent classification. Thus, our overall approach in subset definition and discovery combines the respective strengths of CITRUS and viSNE using two different dimensional reduction methods with different down-sampling approaches to confirm the identification of the identified populations, that is ultimately represented in terms of classic biaxial plots. Finally, to further evaluate the potential pathologic functions of candidate expanded T cell populations, the transcriptional phenotype of the cells will be comprehensively delineated by low cell input RNAseq. This will help define the specific immunobiologic characteristics of these particular cellular subsets that are mechanistic candidates to mediate myocardial inflammation. Detailed transcriptomic analyses of selected immune subpopulations will be generated using a method established to characterize PD-1++CD4+ T cells in the circulation of patients with active, seropositive RA (65).

5.7. Covariates and potential Confounders. Demographics, lifestyle characteristics and non-RA medications will be assessed by structured interview. Resting blood pressure (BP) will be measured 3 times in the seated position, and the average of the last 2 measurements will be used. Hypertension will be defined as a systolic BP of ≥140 mm Hg, a diastolic BP of ≥90 mm Hg, or use of antihypertensive medications. Diabetes will be defined as a fasting serum glucose level of ≥126 mg/dl, a HbA1c >6.4%, or use of antidiabetic medications. Body mass index will be calculated by dividing the patient's weight (in kilograms) by height (in square meters). RA disease duration will be assessed by patient self-report of the date of diagnosis. RA disease activity will be calculated with the Clinical Disease Activity Index (CDAI) and the Disease Activity Score (DAS28) using C-reactive protein (CRP) level (68-70). The Health Assessment Questionnaire (HAQ) will be employed as a measure of self-reported disability (71). Additional questionnaires, including Medical History, Personal History, RA Medications, RA History, and a Visual Analog Scale (VAS) will be used. Current and past use of steroids and disease-modifying anti-rheumatic drugs (DMARDs) will be queried by detailed, examiner-administered questionnaires. Phlebotomy will be performed on the morning of each PET CT scan after overnight fast. Sera and plasma will be separated by centrifugation and frozen at -80^{o}C. Rheumatoid factor will be measured by enzyme-linked immunosorbent assay (ELISA), with seropositivity defined at a level of ≥40 units (IBL America, Minneapolis, MN). Anti–cyclic citrullinated peptide antibody (anti-CCP) will be measured by ELISA (using the CCP3 kit), with

seropositivity defined at a level of ≥60 units (Inova Diagnostics, Woburn, MA). The levels of CRP, interleukin-6 (IL-6), troponins, pro-basic natriuretic peptide (pro-BNP), and lipids will be measured in the Core Laboratory of the Columbia University Medical Center. Comprehensive metabolic panels (CMP) and complete blood counts (CBC) will be collected for standard-of-care hematology and hepato-renal safety.

5.8. Potential Limitations. The exact magnitude of the change in myocardial SUV units that is clinically significant and could translate into improved cardiac outcomes is not known, hence it is possible that the sample size chosen for this proposal under or overestimates it. Similarly, it is conceivable that we do not detect T cell subsets associated with FDG myocardial uptake given the small sample size and the specified panels used to evaluate the T cell subpopulations. Adjusting for potential confounders (i.e., conventional CVD risk factors, CCP Ab, RA disease activity) will also be restricted by our sample size. Additionally, while the nuclear cardiologist will be blinded to treatment assignment, our study is not blinded to treatment arm. If our preliminary data is confirmed with the current proposal, a subsequent larger and blinded randomized controlled trial will be required. Finally, as our proposal focuses on RA patients without clinical cardiovascular disease, the study of the effects of abatacept on RA patients with CVD will need to be explored in a different study.

6. **ELIGIBILITY**

This is a single-center study. Twenty RA patients will be recruited over a planned recruitment period of 24 months, and randomized. The target population consists of patients who are deemed methotrexate-inadequate responders by their treating rheumatologist, and who have not recently received a biologic DMARD. Informed consent will be obtained by the Principal Investigator, co-investigator, or Study Coordinator. Study procedures including screening procedures will not begin until signed informed consent has been obtained.

6.1. Inclusion Criteria: Subjects who meet all of the following criteria are eligible for enrollment into the study:

- Written informed consent signed by the subject.
- Patients age > 18 years.

- Fulfilling the American College of Rheumatology 2010 classification criteria for RA (60).

- 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;
- No recent biologic DMARD treatment defined as prior biologic use other than rituximab in the past 3 months, or rituximab use in the past 6 months.
- If the subject is a woman with childbearing potential, a urine sample will be taken for a pregnancy test. The results of the pregnancy test must be negative.

6.2. Exclusion criteria:

- Prior biologic use other than rituximab in the past 3 months, or rituximab use in the past 6 months.
- Any prior self-reported physician diagnosed CV event (myocardial infarction; angina; stroke or Transient Ischemic Attack (TIA); heart failure; prior CV procedure (i.e., coronary artery bypass graft, angioplasty, valve replacement, pacemaker).
- Active or on-treatment cancer within 5 years of baseline visit
- Prior use of immune checkpoint inhibitors.
- Known pregnancy, HIV, hepatitis B, hepatitis C, active (or untreated latent) tuberculosis.
- Prisoners or subjects who are compulsory detained.

7.RECRUITMENT AND BLINDING

7.1. Recruitment Plan

The target population to be recruited is patients 18 years 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 the rheumatology clinics of Columbia University Medical Center or referred from local rheumatologists. 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.

If the patient is interested in participating, the PI 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.

7.2. Randomization and Blinding

Upon confirmation of subject eligibility, the Study Coordinator will determine treatment assignment through an unblinded 1:1, consecutively, alternating randomization. The nuclear cardiologist reading the FDG-PET/CT scans will be blinded to treatment assignment. 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 nuclear cardiologist and the staff. The nuclear cardiologist and the staff will also be instructed to remind the subject that treatment should not be discussed.

8.RETENTION, AND WITHDRAWAL

8.1. Subject Retention

Once patients are enrolled, a proactive plan for retention will be implemented that includes elements such as regular phone and mailed reminders for each study visit. As commonly used strategies to maximize retention and minimize loss to follow-up, subject relations and subject satisfaction will be emphasized with efforts focusing on congeniality, respectfulness and friendliness in interactions with participants.

8.2. Subject Withdrawal

In this study, all subjects receive active therapy and given the short duration of the trial, we anticipate few to no withdrawals. While great effort will be made to retain subjects in the study, 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 will be recorded. 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. 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:

- Malignancy other than basal or squamous cell
- Repeated subject non-compliance or loss to follow-up
- The subject withdraws consent
- The investigator 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.

9. TREATMENT PLAN AND STUDY DRUGS

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 determination that the subject is eligible for inclusion in the trial, the subject will be randomized to trial treatment. Once a subject has been assigned to treatment, the appropriate treatment will be provided. Subjects will begin their treatment arm on the date of the baseline visit.

As the study drugs are FDA approved, they will be stored in accordance with the package insert in a secure area and under the appropriate environmental conditions. A disposition record of both abatacept (supplied by BMS) and adalimumab, will be maintained where these drugs will be inventoried and dispensed. Procedures for proper disposal of drug when appropriate will take place in accordance to institutional regulations and procedures.

9.1. Description of Treatment Medications

A brief description of each of the study medications (all of which 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) Abatacept: https://packageinserts.bms.com/pi/pi_orencia.pdf
3) Methotrexate Injection: http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/011719s117lbl.pdf
4) Methotrexate tablets: http://www.rheumatrex.info/pdf/RheumatrexPackageInsert.pdf

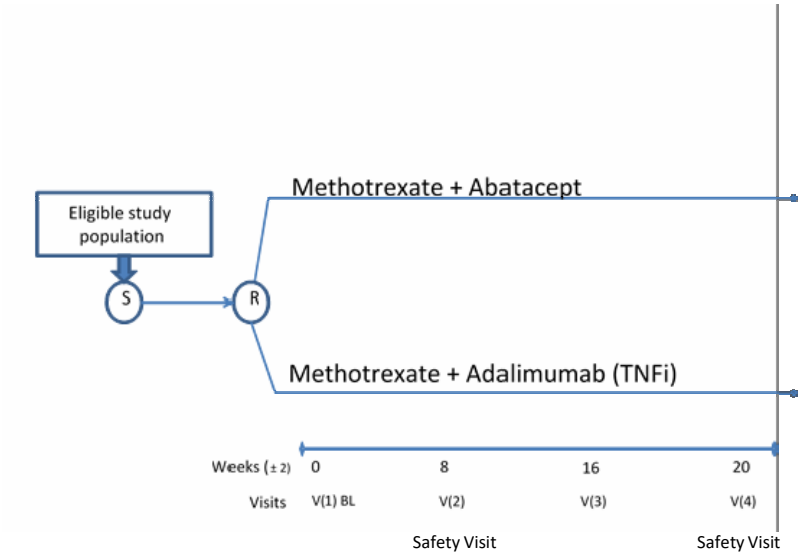
Adalimumab. The standard initial dose of adalimumab is 40 mg SC once every other week. It is provided already diluted to the appropriate concentration in pre-filled syringes. It is also injected into the abdomen or thigh and must be refrigerated.

Abatacept. Treatment with abatacept will consist of weekly subcutaneous (SQ) injections at a dose of 125mg. Similar to adalimumab, it is provided already diluted to the appropriate concentration in pre-filled syringes and autoinjector pen, and also injected into the abdomen or thigh and must be refrigerated. *At the end of the study period, Bristol-Myers Squibb Company will not continue to*

supply study drug to subjects/investigators unless the Sponsor-Investigator chooses to extend their study. The investigator is responsible to ensure that the subject receives appropriate standard of care or other appropriate treatment in the independent medical judgement of the Investigator to treat the condition under study.

9.2. Treatment Algorithm

Figure 3.



Subjects will be randomized in an unblinded, 1:1, consecutive, alternating randomization to treatment with adalimumab 40 mg SQ every other week or abatacept 125 mg SQ weekly, each in addition to concomitant MTX. Subjects will remain on this new medication until the end of the treatment at 16 weeks.

9.3. Concomitant Medications.

Corticosteroids. Patients who are not receiving corticosteroids and those receiving low dose steroids (≤ 15 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 FDG uptake independently of the randomized treatment. No oral corticosteroid "bursts" will be allowed

unless there is reason that an intra-articular injection is not possible. If a burst is required, it can be no more than 15mg per day (prednisone equivalent) at maximum and no longer than 14 days in duration. Corticosteroid bursts cannot occur within 14 days of either of the FDG PET/CT scans.

10.SCHEDULE OF ASSESSMENTS

Consent will be obtained at the beginning of the Screening/baseline visit (Visit 1). Patients who meet eligibility criteria and provide informed consent will be considered enrolled and will undergo the additional 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). Subjects found to have active hepatitis or active infection (including tuberculosis) will be excluded. If the required screening blood work was completed by the subject within 2 weeks of the screening visit, 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/baseline visit, then safety labs performed during those 8 weeks do not need to be repeated at the screening/baseline visit. If these additional laboratory- and imaging-based eligibility criteria are met and eligibility is confirmed, the cardiac FDG PET/CT scan will be scheduled. For the cardiac 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 into the study. The subject will complete additional questionnaires at baseline. An interim/safety visit at 8 weeks will take place, followed by a final visit at week 16. Blood will be drawn at each visit. A repeat cardiac FDG PET-CT scan will be performed at 16 weeks. An additional safety visit at week 20 will be performed to assess potential adverse events.

Treatment drug will be dispensed at the baseline and the week 8 study visits.

Table 1.

Study Procedures	Baseline	8 weeks (± 2)	16 weeks (± 2)	20 weeks (± 2)

Eligibility/ Informed consent	X			
Demographics, medical history	X			
Conventional CVD risk factors	X	X	X	
Physical Exam	X	X	X	X
Cardiac FDG PET CT	X		X	
RA Activity Assessment[1]	X	X	X	X
T cell immunophenotyping	X		X	
Laboratory assessments[2,3,4]	X	X	X	X
Study Drug dispensation	X	X		
Adverse Events Assessment		X	X	X
[1] CDAI, DAS-28. [2] CRP, ESR, IL-6, CCP Ab, RF, hemoglobin A1c, lipid panel, troponins, pro-BNP, CMP, CBC. [3] Hepatitis B and C, quantiferon gold TB: only performed at baseline. [4] Pregnancy test if applicable.				

11.**SAFETY REPORTING**

11.1. Adverse Events

11.1.1. 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.

11.1.2. Serious Adverse Event

A serious adverse event (SAE) is defined as any AE which:

- Results in death

- Is life-threatening (refers as any event in which the patient was at risk of death at the time of the event, it does not refer to an event that hypothetically might have caused death if more severe)
- 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.

- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.

Although pregnancy, overdose, potential drug-induced liver injury (DILI), and cancer are not always serious by regulatory definition, these events will be handled as SAEs.

Any component of a study endpoint that is considered related to study therapy will be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

The following hospitalizations will not be considered SAEs in agreement with the PI and BMS clinical studies:

A visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event).

Elective surgery, planned prior to signing consent.

Admissions as per protocol for a planned medical/surgical procedure.

Routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy).

Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases.

Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols).

11.1.3. Relationship of an Adverse Event to Study Intervention

Medical judgment should be used to assess the causal relationship of an event to study intervention considering all relevant factors, 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.

11.1.4. Severity of an Adverse Event

The following scale will be used to grade adverse events:

1. Mild: Awareness of sign(s) or symptom(s) that is/are easily tolerated; no intervention required; no impact on activities of daily living (ADL)
2. Moderate: local, or non-invasive intervention indicated; enough discomfort or moderate impact on ADL
3. Severe: significant symptoms requiring invasive intervention or causing inability to work or to performed ADL; subject seeks medical attention, needs major assistance with ADL.

11.2. 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.

11.3. Reporting of Adverse Events, Serious Adverse Events, and Unanticipated Problems

The principal investigator and/or research staff will be monitoring for any possible adverse event/serious adverse event or unanticipated events at every study visit and/or throughout the subject participation in the study. Any AE will be documented in a study AE/SAE log with paper copies of any supplemental records that support the event, the PI will review the event and follow up as appropriate. All adverse events that are anticipated or unrelated will be kept in a AE log, reviewed by the PI and will be submitted regularly to the local IRB according to the local standard of procedure. All adverse event that are unanticipated and related or possibly related to the research and all Serious Adverse Events (SAEs) (regardless of expectedness, relatedness, or if they meet the definition for unanticipated problems) will be reported promptly within 24-48 hours from the PI receiving the notification of the event and the PI will be responsible to secure that the local IRB is promptly notified. Participants that sign a consent form will be considered enrolled until their participation is terminated or they withdraw consent. AEs/SAE/UE will be reported on all those enrolled.

All SAE should be followed up and 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 principal investigator and/or co-investigators should be provided in the report, 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 and FDG PET-CT protocol. Those adverse events that occur, which are not specified on the package inserts or expected as per 18F-FDG PET-CT guidelines, will be considered unanticipated problems and will require reporting to the principal investigator and the IRB.

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 24-48 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 an Adverse Event Form, which will also be used to track the AE/SAE. AEs 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 research staff will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 14 days (for SAEs) after the last day of study participation. At each study visit, the investigator and/or research staff will inquire about the occurrence of AE/SAEs since the last visit

Some incidental findings are likely to be identified in the PET/CT scans, or at other points throughout the study. The PI will be responsible for communicating any incidental findings to the participant's primary care physician (PCP) or Rheumatologist. 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 or Rheumatologist should occur within 24-48 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 (PCP) or Rheumatologist. The PI will 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.

11.3.1 Reporting to BMS

- All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through the last study visit on week 20, will be reported to BMS Worldwide Safety, whether related or not related to study drug.
- Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, will be collected, including those thought to be associated with protocol-specified procedures. The investigator will report any SAE occurring after these aforementioned time periods, which is believed to be related to study drug or protocol-specified procedure.
- An SAE report will be completed for any event where doubt exists regarding its seriousness;
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship will be specified in the SAE Report Form.

The CIOMS SAE form (http://www.cioms.ch/index.php/cioms-form-i) will be used to report SAEs and pregnancies to BMS via email: Worldwide.Safety@bms.com, aepbusinessprocess@bms.com, or fax: +1 609-818-3804. The BMS Protocol number will be included on the SAE form or on the cover sheet with the SAE form transmission.

- In accordance with local regulations, BMS will notify investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (ie, not previously described in the IB). An event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Investigator notification of these events will be in the form of a SUSAR Report.
- Other important findings which may be reported by BMS as an Expedited Safety Report (ESR) include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (eg, animal) study, important safety recommendations from a

study data monitoring committee, or sponsor decision to end or temporarily halt a clinical study for safety reasons.

- Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.
- In addition to the Sponsor Investigator's responsibility to report events to their local HA, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports). SAEs, whether related or not related to study drug, and pregnancies will be reported to BMS within 1-2 business days of becoming aware of the event. If only limited information is initially available, follow-up reports will be submitted. If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report will be sent within 1-2 Business Days to BMS using the same procedure used for transmitting the initial SAE report. All SAEs should be followed through the duration of the study.

11.4. Pregnancy

If the subject is a woman with childbearing potential, a urine sample will be taken for a pregnancy test from baseline through week 20, especially prior any study procedure at baseline and week 16. The results of the pregnancy test must be negative. Due to the possible teratogenic effect of the "radiation", "medication", highly effective contraception has to be performed and documented negative pregnancy test on the date of the study visit/s.

In the rare case that a female subject participating in the study becomes pregnant after study intervention, the research staff must immediately report the event to the principal investigator and a serum pregnancy test should be performed. The PI should bring the subject for an evaluation, a written report and pregnancy form should follow as well as periodically follow up to monitor the outcome of the subject's pregnancy. The pregnancy event should be reported to the

IRB within 24-48hrs of confirming the event. A copy of the report should be kept in the subject study binder.

The following delineates specific responsibilities of staff members:

- The **Research Coordinator** will complete the Adverse Event Form (and Unanticipated Problems on the corresponding form); 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 **Principal Investigator** will confirm that all AEs and UPs are correctly documented by the coordinator; be available to answer any questions that the coordinators may have concerning AEs; The investigator together with her/his research staff will notify Worldwide.Safety@bms.com of this event via the CIOMS SAE form.

- Protocol-required procedures for study discontinuation and follow-up will be performed on the participant.

- Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the CIOMS, MedWatch, BMS Pregnancy Surveillance Form, **or** approved site SAE form. A BMS Pregnancy Surveillance Form may be provided upon request. will be provided through the duration of the study.

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

11.5. NONSERIOUS ADVERSE EVENT

- Non-serious Adverse Events (AE) will be provided to BMS as part of an annual reporting requirement.

A ***non-serious adverse event*** is an AE not classified as serious.

Non-serious Adverse Event Collection and Reporting

The collection of non-serious AE information will begin at initiation of study drug. All non-serious adverse events (not only those deemed to be treatment-related) will be collected continuously during the treatment period and for a minimum of 30 days following the last dose of study treatment.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

11.6. Laboratory Test Abnormalities

All laboratory test results captured as part of the study will be recorded following institutional procedures. Test results that constitute SAEs will be documented and reported to BMS as such at the discretion of the PI in accordance with standard of care practice.

The following laboratory abnormalities will be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (eg, anemia versus low hemoglobin value).

11.7. **Potential Drug Induced Liver Injury (DILI)**

Specific criteria for identifying potential DILI have not been identified for this protocol. Standard medical practice in identifying and monitoring hepatic issues will be followed.

11.8. Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose will be reported as an SAE.

11.9. Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, X-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

12. STATISTICAL ANALYSIS

12.1. Overview. This is a pilot study to evaluate the changes in FDG myocardial uptake post-biologic treatment of RA patients with abatacept vs adalimumab. Summary statistics for outcomes and predictor variables will be examined; with comparisons made using student t-test and Wilcoxon rank-sum test for normally and non-normally distributed continuous variables, respectively. Counts and percentages will be calculated for categorical variables and compared using the chi-square or Fisher's exact test, as appropriate. In Aim 1, using generalized estimating equations (GEE), models will be constructed to explore the association between treatment arms (abatacept vs adalimumab) and change in myocardial FDG uptake treated as a continuous variable (measured by SUV units). Similarly, linear mixed effects models will be used to define the association between T cell subpopulations and myocardial FDG uptake per treatment arm strata. Given the small sample size of the study, we will not have the power to adjust for multiple variables, but the two treatment arms will be matched for age, sex, and disease activity, and the contribution of RA-disease characteristics and traditional CVD risk factors to the variability of myocardial FDG uptake will be ascertained. All statistical calculations will be performed using SAS 9.4. In all tests, a 2-tailed α of 0.05 will define statistical significance.

12.2. Sample Size and Power Analysis. Data on what is considered a significant change in myocardial FDG uptake in RA is not yet known. Hence, based on our preliminary data that included only 8 patients on abatacept, and as a proof of concept, for the proposed pilot

studies we will include 10 patients in the abatacept arm and compare them with 10 patients on adalimumab. Through normal approximation using the Z statistic, with a SD of 0.5 in the SUV units of myocardial FDG uptake seen in the RHYTHM study, we will have 80% power to detect an effect size of 0.6 between the groups, at an alpha level of 0.05.

13. OTHER AND ADMINISTRATIVE CONSIDERATIONS

13.1. Data Management

For data collection and management purposes, subjects will be identified by a subject number only. All collected data will be transferred to a database exclusively using the SID only. The raw information for input into the database will be stored in binders in a locked cabinet in the PI's office. The database will be saved in an encrypted computer and backed up onto an encrypted portable hard drive on a weekly basis, and the backups also kept in the locked cabinet. Hard-copy files will be stored in a locked cabinet in a locked room. Only the Principal Investigator and study staff will have access to these files. Those with access are held to the strictest standards of confidentiality. Data reported on the database must be consistent with the source data or the discrepancies must be explained. When needed, copies of supported documentation such as medical records current and/or previous should be requested and filed prior de-identification. All data must be derived from source documents.

13.2. Confidentiality

The confidentiality of participating subjects will be maintained. Subjects will be individually assigned a unique coded identification number The data file and the file that links the unique subject number to patient identity will be maintained separately in password protected files on password protected and fully encrypted computers in a locked office in the Division of Rheumatology. Hard-copy files will be stored in a locked cabinet in a locked room. Those with access are held to the strictest standards of confidentiality. Documents that identify the subject beyond subject number will not be released outside of Columbia University Medical Center and/or New York-Presbyterian Hospital (e.g., the signed informed consent document) and will be maintained in strict confidence by the investigator, except to the extent necessary to allow auditing by the regulatory authorities, study monitor, or sponsor representatives.

Standard HIPAA procedures and guidelines will be enforced. The consent form discloses to potential participants the individuals and/or agencies that are able to look at and copy research records, namely:

- The principal investigator, co-investigators, study staff and other medical professionals who may be evaluating the study

- Authorities from Columbia University and New York-Presbyterian Hospital, including the Institutional Review Board ('IRB')

- The Office of Human Research Protections ('OHRP')

- Representatives from and or of the FDA, federal or state government agencies, other health authorities and their commercial partners.

- Information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws. In addition to confidentiality disclosures described in the consent form, potential participants will also sign the attached study specific HIPAA Form before participating in this study. The consent discussion will take place in private locations (e.g. interview room; physician's office; exam room). Participants will be given an ICF including the HIPAA form providing a specific description of the ways in which their personal health information will be used and disclosed in the course of this study. The Investigators or Research staff will explain and answer all the questions participants may have regarding their privacy protection. If they decide to participate, participants will be asked to sign the combined consent and HIPAA form authorizing the use and disclosure of their personal information before starting the study.

13.3. Potential Risk

A risk exists that despite all provisions to maintain participant's confidentiality, confidentiality nonetheless may be compromised. As mentioned above every effort will be made to secure the data collected and separate identifying from de-identified information.

Phlebotomy is associated with minor discomfort and occasional patients may experience a more severe vaso-vagal reaction and light-headedness. A minor risk of a skin infection at the site of phlebotomy also exists but may be treated with a short course of antibiotics should it occur.

The mortality of the PET-CT imaging is low and associated mainly with the administration of adenosine/regadenoson for evaluations under stress. In patients with established coronary artery disease, mortality is reported to be 2/10000patients. The risk for an acute myocardial infarction is also reported to be 2/10000. These numbers will not be applicable to our participants as candidates with prior cardiovascular events will be screened out. Furthermore, the mortality at the Columbia University PET center has been 0% for PET-CT stress tests for the last 15 years with over 11000 PET-CT stress tests performed.

The radiation exposure/dosimetry with cardiac PET with: F18 FDG imaging is within FDA guidelines for research of this type. The radiation obtained during the PET CT-F18-FDG imaging examinations for this protocol would be 16.46 mSvs (1646 mrem) which is equivalent to about 5.4 years of natural and cosmic radiation. The patients will have to be fasting overnight when they present for their study visits and this may cause mild discomfort.

In addition, study participants will be asked if they received radiation from imaging procedures outside the current protocol (for their regular clinical care). If other imaging studies have taken place, the cumulative radiation exposure will be calculated and if it exceeds the level of 50 mSv which is considered to be the maximum acceptable radiation exposure per year then the subject will not participate in the study.

The testing performed in this study may unmask conditions that have been silent (asymptomatic). If a significant medical finding is noted during participation in this study, the PI will be notified immediately by the nuclear cardiologist, study personnel or the PET-CT center.

13.4. Potential Benefit

There are no direct benefits anticipated for the participating subjects. However, evaluating RA patients for early and potentially cardiac reversible changes such as the presence of myocardial inflammation will offer insight into the pathophysiology driving this disorder and guide an early intervention when warranted. The findings derived from this study could benefit the society by having multidisciplinary implications that could lead to the prevention of clinical vascular events and improvement in survival in patients with RA.

13.5. Records Retention

Source documents provide evidence of the patient/subject existence and validate the integrity of the data collected. The trial site must retain the source documents, supported materials and any essential documents for as long as the principal investigator considers necessary.

13.6. Publication Strategy

The results of this research may be published in scientific journals or presented at medical meetings, but patient identity will not be disclosed.

14. REFERENCE LIST

1. Symmons DP, Jones MA, Scott DL, Prior P. Long-term mortality outcome in patients with rheumatoid arthritis: early presenters continue to do well. J Rheumatol. Jun 1998;25(6):1072-1077.
2. Solomon DH, Karlson EW, Rimm EB, Cannuscio CC, Mandl LA, Manson JE, et al. Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis. Circulation 2003;107:1303–7.
3. Sihvonen S, Korpela M, Laippala P, Mustonen J, Pasternack A. Death rates and causes of death in patients with rheumatoid arthritis: a population-based study. Scand J Rheumatol 2004;33:221–7.
4. Solomon DH, Goodson NJ, Katz JN, Weinblatt ME, Avorn J, Setoguchi S, et al. Patterns of cardiovascular risk in rheumatoid arthritis. Ann Rheum Dis 2006;65:1608–12.
5. Wolfe F, Freundlich B, Straus WL. Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis. J Rheumatol 2003;30:36–40.
6. Nicola PJ, Maradit-Kremers H, Roger VL, Jacobsen SJ, Crowson, CS, Ballman KV, et al. The risk of congestive heart failure in rheumatoid arthritis: a population-based study over 46 years. Arthritis Rheum 2005;52:412–20.
7. Nicola PJ, Crowson CS, Maradit-Kremers H, Ballman KV, Roger VL, Jacobsen SJ, et al. Contribution of congestive heart failure and ischemic heart disease to excess mortality in rheumatoid arthritis. Arthritis Rheum 2006;54:60.
8. Wolfe F, Michaud K. Heart failure in rheumatoid arthritis: rates, predictors, and the effect of anti-tumor necrosis factor therapy. Am J Med 2004;116:305–11.
9. Lebowitz WB. Heart in Rheumatoid Arthritis (Rheumatoid Disease) - A Clinical and Pathological Study of 62 Cases. Ann Intern Med. 1963; 58(1):102.
10. Cathcart ES, Spodick DH. Rheumatoid heart disease. A study of the incidence and nature of cardiac lesions in rheumatoid arthritis. N Engl J Med, 1962. 266: p. 959-64.
11. Sokoloff L. Cardiac Involvement in Rheumatoid Arthritis and Allied Disorders: Current Concepts. Mod Concepts Cardiovasc Dis, 1964. 33: p. 847-50.
12. Grundtman C, et al., Cardiovascular disease in patients with inflammatory rheumatic disease is associated with up-regulation of markers of inflammation in cardiac microvessels and cardiomyocytes. Arthritis and rheumatism, 2010. 62(3): p. 667-673.
13. Bennett MK, et al. Evaluation of the role of endomyocardial biopsy in 851 patients with unexplained heart failure from 2000-2009. Circ Heart Fail, 2013. 6(4): p. 676-84.

14. Uemura A. et al. Histologic diagnostic rate of cardiac sarcoidosis: evaluation of endomyocardial biopsies. Am Heart J, 1999. 138(2 Pt 1): p. 299-302.

15. Han B, Jiang H, Liu Z, Zhang Y, Zhao L, Xi J, et al. CTLA4-Ig relieves inflammation in murine models of coxsackievirus B3-induced myocarditis. Can J Cardiol. 2012 Mar-Apr;28(2):239-44.

16. Matsui Y, Inobe M, Okamoto H, Chiba S, Shimizu T, Uede T, et al.Blockade of T cell costimulatory signals using adenovirus vectors prevents both the induction and the progression of experimental autoimmune myocarditis. J Mol Cell Cardiol. 2002 Mar;34(3):279-95.

17. Abe S, Hanawa H, Hayashi M, Yoshida T, Komura S, Aizawa Y. Prevention of experimental autoimmune myocarditis by hydrodynamics-based naked plasmid DNA encoding CTLA4-Ig gene delivery. J Card Fail. 2005 Sep;11(7):557-64.

18. Liu W1, Gao C, Zhou BG, Li WM. Effects of adenovirus-mediated gene transfer of ICOSIg and CTLA4Ig fusion protein on experimental autoimmune myocarditis. Autoimmunity. 2006 Mar;39(2):83-92.

19. Johnson DB, Balko JM, Compton ML, Chalkias S, Gorham J, Moslehi JJ, et al. Fulminant Myocarditis with Combination Immune Checkpoint Blockade. N Engl J Med. 2016 Nov 3;375(18):1749-1755.

20. Varricchi G, Galdiero MR, Tocchetti CG. Cardiac Toxicity of Immune Checkpoint Inhibitors: Cardio-Oncology Meets Immunology. Circulation. 2017 Nov 21;136(21):1989-1992.

21. Westhovens R, Robles M, Ximenes AC, et al. Clinical efficacy and safety of abatacept in MTX-naive patients with early rheumatoid arthritis and poor prognostic factors. Ann Rheum Dis 2009;68:1870-1877.

22. Moreland LW, Alten R, Van den Bosch F, et al. Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4Ig and LEA29Y eighty-five days after the first infusion. Arthritis Rheum 2002;46:1470-1479.

23. Kremer JM, Westhovens R, Leon M, et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. N Engl J Med 2003;349:1907-1915.

24. Weinblatt M, Combe B, Covucci A, et al. Safety of the selective costimulation modulator abatacept in RA patients receiving background biologic and nonbiologic DMARDs: a one-year randomized, placebo-controlled study. Arthritis Rheum 2006;54:2807-2816.

25. Genovese MC, Becker JC, Schiff M, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. N Engl J Med 2005;353:1114-1123.

26. Bluestone JA1, St Clair EW, Turka LA. CTLA4Ig: bridging the basic immunology with clinical application. Immunity. 2006 Mar;24(3):233-8.

27. Pieper J, Herrath J, Raghavan S, Muhammad K, Vollenhoven Rv, Malmström V.CTLA4-Ig (abatacept) therapy modulates T cell effector functions in autoantibody-positive rheumatoid arthritis patients. BMC Immunol. 2013 Aug 5;14:34.

28. Bonelli M, Göschl L, Blüml S, Karonitsch T, Hirahara K, Ferner E, Steiner CW, Steiner G, Smolen JS, Scheinecker C. Abatacept (CTLA-4Ig) treatment reduces T cell apoptosis and regulatory T cell suppression in patients with rheumatoid arthritis. Rheumatology (Oxford). 2016 Apr;55(4):710-20.

29. Buch MH, Boyle DL, Rosengren S, Saleem B, Reece RJ, Rhodes LA, Radjenovic A, English A, Tang H, Vratsanos G, O'Connor P, Firestein GS, Emery P. Mode of action of abatacept in rheumatoid arthritis patients having failed tumour necrosis factor blockade: a histological, gene expression and dynamic magnetic resonance imaging pilot study. Ann Rheum Dis. 2009 Jul;68(7):1220-7.

30. Kobayashi Y, et al. Assessment of myocardial abnormalities in rheumatoid arthritis using a comprehensive cardiac magnetic resonance approach: a pilot study. Arthritis Res Ther, 2010. 12(5): p. R171.

31. Kobayshi H, et al. Magnetic Resonance-Detected Myocardial Inflammation and Fibrosis in Rheumatoid Arthritis: Associations of Disease Characteristics and N-terminal pro Brain Natriuretic Peptide Levels. Arthritis care & research, 2016.

32. Ntusi NA, et al. Diffuse Myocardial Fibrosis and Inflammation in Rheumatoid Arthritis: Insights From CMR T1 Mapping. JACC. Cardiovascular imaging, 2015. 8(5): p. 526-536.

33. Holmström M, et al. Cardiac magnetic resonance imaging reveals frequent myocardial involvement and dysfunction in active rheumatoid arthritis. Clinical and experimental rheumatology, 2016. 34(3): p. 416-423.

34. Tang R, et al. Impact of Patient Preparation on the Diagnostic Performance of 18F-FDG PET in Cardiac Sarcoidosis: A Systematic Review and Meta-analysis. Clin Nucl Med, 2016. 41(7): p. e327-39.

35. Okumura W, et al. Usefulness of fasting 18F-FDG PET in identification of cardiac sarcoidosis. J Nucl Med, 2004. 45(12): p. 1989-98.

36. Ozawa K, et al. Determination of optimum periods between onset of suspected acute myocarditis and (1)(8)F-fluorodeoxyglucose positron emission tomography in the diagnosis of inflammatory left ventricular myocardium. Int J Cardiol, 2013. 169(3): p. 196-200.

37. Hiari N, Rudd JH. FDG PET imaging and cardiovascular inflammation. Curr Cardiol Rep, 2011. 13(1): p. 43-8.
38. Maki-Petaja KM, et al. Anti-Tumor Necrosis Factor-alpha Therapy Reduces Aortic Inflammation and Stiffness in Patients With Rheumatoid Arthritis. Circulation, 2012. 126(21): p. 2473-80.
39. Ahmadian A, et al. Quantitative interpretation of FDG PET/CT with myocardial perfusion imaging increases diagnostic information in the evaluation of cardiac sarcoidosis. J Nucl Cardiol, 2014. 21(5): p. 925-39.
40. Osborne MT, et al. Reduction in (1)(8)F-fluorodeoxyglucose uptake on serial cardiac positron emission tomography is associated with improved left ventricular ejection fraction in patients with cardiac sarcoidosis. J Nucl Cardiol, 2014. 21(1): p. 166-74.
41. Yokoyama R, et al. Quantitative analysis of myocardial 18F-fluorodeoxyglucose uptake by PET/CT for detection of cardiac sarcoidosis. International journal of cardiology, 2015. 195: p. 180-187.
42. Garedew A, Henderson SO, Moncada S. Activated macrophages utilize glycolytic ATP to maintain mitochondrial membrane potential and prevent apoptotic cell death. Cell Death Differ, 2010. 17(10): p. 1540-50.
43. Kubota R, et al. Microautoradiographic study for the differentiation of intratumoral macrophages, granulation tissues and cancer cells by the dynamics of fluorine-18-fluorodeoxyglucose uptake. J Nucl Med, 1994. 35(1): p. 104-12.
44. Lee WW, et al. PET/MRI of inflammation in myocardial infarction. J Am Coll Cardiol, 2012. 59(2): p. 153-63.
45. Maya Y, et al. 11C-methionine PET of myocardial inflammation in a rat model of experimental autoimmune myocarditis. J Nucl Med, 2016.
46. Podack ER. Execution and suicide: cytotoxic lymphocytes enforce draconian laws through separate molecular pathways. Curr Opin Immunol, 7 (1995), p. 11-16.
47. Yu DT, Winchester RJ, Fu SM, Gibofsky A, Ko HS, Kunkel HG. Peripheral blood Ia-positive T cells. Increases in certain diseases and after immunization. J Exp Med. 1980;151(1):91-100.
48. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol. 2004;22:745-63.
49. Schmidt D, Goronzy JJ, Weyand CM. CD4+ CD7- CD28- T cells are expanded in rheumatoid arthritis and are characterized by autoreactivity. The Journal of clinical investigation. 1996;97(9):2027-37.

50. van Bergen J, Thompson A, van der Slik A, Ottenhoff TH, Gussekloo J, Koning F. Phenotypic and functional characterization of CD4 T cells expressing killer Ig-like receptors. Journal of Immunology. 2004;173(11):6719-26.

51. Appay V. The physiological role of cytotoxic CD4(+) T-cells: the holy grail? Clinical and Experimental Immunology. 2004;138(1):10-3.

52. Morishita Y, Sao H, Hansen JA, Martin PJ. A distinct subset of human CD4+ cells with a limited alloreactive T cell receptor repertoire. Journal of Immunology. 1989;143(9):2783-9.

53. Nakajima T, Goek O, Zhang X, Kopecky SL, Frye RL, Goronzy JJ, et al. De novo expression of killer immunoglobulin-like receptors and signaling proteins regulates the cytotoxic function of CD4 T cells in acute coronary syndromes. Circulation research. 2003;93(2):106-13.

54. Schmidt D, Martens PB, Weyand CM, Goronzy JJ. The repertoire of CD4+ CD28- T cells in rheumatoid arthritis. Mol Med 1996;2:608–18.

55. Pawlik A, Ostanek L, Brzosko I, Brzosko M, Masiuk M, Machalinski B, et al. The expansion of CD4+CD28- T cells in patients with rheumatoid arthritis. Arthritis Res Ther 2003;5:R210–3.

56. Maeda T, Yamada H, Nagamine R, Shuto T, Nakashima Y, Hirata G, et al. Involvement of CD4+,CD57+ T cells in the disease activity of rheumatoid arthritis. Arthritis Rheum 2002;46:3 79–84.

57. Imberti L, Sottini A, Signorini S, Gorla R, Primi D. Oligoclonal CD4+ CD57+ T-cell expansions contribute to the imbalanced T-cell receptor repertoire of rheumatoid arthritis patients. Blood 1997;89:2822—32.

58. Wang EC, Lawson TM, Vedhara K, Moss PA, Lehner PJ, Borysiewicz LK. CD8 high+ (CD57+) T cells in patients with rheumatoid arthritis. Arthritis Rheum 1997;40:237–48.

59. Jabri B, Selby JM, Negulescu H, Lee L, Roberts AI, Beavis A, et al. TCR specificity dictates CD94/NKG2A expression by human CTL [published erratum appears in Immunity 2002;17:835]. Immunity 2002;17:487–99.

60. Aletaha D, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum, 2010. 62(9): p. 2569-81.

61. Osborne MT, et al. Patient preparation for cardiac fluorine-18 fluorodeoxyglucose positron emission tomography imaging of inflammation. Journal of nuclear cardiology : official publication of the American Society of Nuclear Cardiology, 2017. 24(1): p. 86-99.

62. Demeure F, et al. A randomized trial on the optimization of 18F-FDG myocardial uptake suppression: implications for vulnerable coronary plaque imaging. J Nucl Med, 2014. 55(10): p. 1629-35.

63. Cerqueira MD, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Circulation, 2002. 105(4): p. 539-542.

64. Amir el AD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, et al. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. Nat Biotechnol. 2013;31(6):545-52.

65. Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nature. 2017;542(7639):110-4.

66. Winchester R, Wiesendanger M, O'Brien W, Zhang HZ, Maurer MS, Gillam LD, et al. Circulating activated and effector memory T cells are associated with calcification and clonal expansions in bicuspid and tricuspid valves of calcific aortic stenosis. J Immunol 2011;187:1006–14.

67. Bruggner RV, Bodenmiller B, Dill DL, Tibshirani RJ, Nolan GP. Automated identification of stratifying signatures in cellular subpopulations. Proc Natl Acad Sci U S A. 2014;111(26):E2770-7.

68. Inoue E, et al. Comparison of Disease Activity Score (DAS)28- erythrocyte sedimentation rate and DAS28- C-reactive protein threshold values. Ann Rheum Dis, 2007. 66(3): p. 407-9.

69. Prevoo MLL, et al. Modified disease activity scores that include twenty-eight-joint counts development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis & Rheumatism, 1995. 38(1): p. 44-48.

70. Wells G, et al. Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. Annals of the Rheumatic Diseases, 2009. 68(6): p. 954-960.

71. Fries JF, et al. Measurement of patient outcome in arthritis. Arthritis and rheumatism, 1980. 23(2): p. 137-145.