Regulatory T-Cell Response to Low-Dose Interleukin-2 in Ischemic Heart Disease

Tian X. Zhao, Ph.D.1, Rouchelle S. Sriranjan, M.B.Ch.B.1, Zewen Kelvin Tuong, Ph.D.2,3, Yuning Lu, Ph.D.1, Andrew P. Sage, Ph.D.1, Meritxell Nus, Ph.D.1, Annette Hubsch, B.Sc.4, Fotini Kaloyirou, Ph.D.4, Evangelia Vamvaka, M.Sc.4, Joanna Helmy, B.Sc.4, Michalis Kostapanos, Ph.D.4, Navazh Jalaludeen4, David Klatzmann, M.D.5, Alain Tedgui, Ph.D.6, James H.F. Rudd, Ph.D.7, Sarah J. Horton, Ph.D.7,8, Brian J.P. Huntly, Ph.D.7,8, Stephen P. Hoole, D.M.9, Simon P. Bond, Ph.D.10, Menna R. Clatworthy, Ph.D.2,3,11, Joseph Cherian, M.B.Ch.B.4,10, and Ziad Mallat, M.D.1,6

Dr. Clatworthy, Dr. Cherian, and Dr. Mallat contributed equally to this article.

Abstract

BACKGROUND Atherosclerosis is a chronic inflammatory disease of the artery wall. Regulatory T cells (Tregs) limit inflammation and promote tissue healing. Low doses of interleukin (IL)-2 have the potential to increase Tregs, but its use is contraindicated for patients with ischemic heart disease.

METHODS In this randomized, double-blind, placebo-controlled, dose-escalation trial, we tested low-dose subcutaneous aldesleukin (recombinant IL-2), given once daily for 5 consecutive days. In study part A, the primary end point was safety, and patients with stable ischemic heart disease were randomly assigned to receive placebo or to one of five dose groups (range, 0.3 to 3.0 × 10^6 IU daily). In study part B, patients with acute non-ST elevation myocardial infarction or unstable angina were randomly assigned to receive placebo or to one of two dose groups (1.5 and 2.5 × 10^6 IU daily). The coprimary end points were safety and the dose required to increase circulating Tregs by 75%. Single-cell RNA-sequencing of circulating immune cells was used to provide a mechanistic assessment of the effects of aldesleukin.

RESULTS Forty-four patients were randomly assigned to either study part A (n=26) or part B (n=18). In total, 3 patients withdrew before dosing, 27 received active treatment, and 14 received placebo. The majority of adverse events were mild. Two serious adverse events occurred, with one occurring after drug administration. In parts A and B, there was a dose-dependent increase in Tregs. In part B, the estimated dose to achieve a 75% increase in Tregs was 1.46 × 10^6 IU (95% confidence interval, 1.06 to 1.87). Single-cell RNA-sequencing demonstrated the engagement of distinct pathways and cell-cell interactions.

CONCLUSIONS In this phase 1b/2a study, low-dose IL-2 expanded Tregs without adverse events of major concern. Larger trials are needed to confirm the safety and to further evaluate the efficacy of low-dose IL-2 as an anti-inflammatory therapy for patients with ischemic heart disease.
Introduction

Atherosclerosis is a chronic inflammatory condition. Myocardial infarction (MI) triggers an acute immune response that accelerates atherosclerosis and can contribute to the progression of heart failure. Studies of canakinumab and colchicine have shown that modulating inflammation can improve outcomes for patients with coronary disease; however, despite these advances, there currently are no targeted immunomodulatory therapies approved for use in this disease setting.

Regulatory T cells (Tregs) control immune activation and enforce tolerance. Some Tregs reside in non-lymphoid tissues where they maintain tissue homeostasis and control sterile inflammation. In preclinical models, Treg deficiency accelerated atherosclerosis, whereas Treg supplementation was atheroprotective. In preclinical models of MI, Tregs were important in promoting myocardial healing. Observational studies have shown a decrease in the number and function of circulating Tregs in patients with MI, whereas the expansion and activation of proinflammatory effector T cells (Teffs) was positively correlated with the occurrence of ischemic heart disease.

Interleukin-2 (IL-2) at high doses stimulates Teffs and is an approved cancer treatment. Paradoxically, low-dose IL-2 (doses a thousand-fold lower than those used for oncologic indications) can selectively activate Tregs, enabling their expansion without increasing Teffs. Aldesleukin (recombinant IL-2) at low doses has been used in small early phase clinical trials in patients with autoimmune diseases; however, its use is contraindicated in patients with a history, or current evidence of, severe cardiac disease, primarily owing to the risk of capillary leak syndrome, pulmonary edema, and tachyarrhythmias associated with high-dose intravenous regimens.

Therefore, we conducted a phase 1b/2a trial to assess the safety of multiple ascending low doses of aldesleukin in patients with stable ischemic heart disease and acute coronary syndromes (ACS) and to determine a dose that selectively and substantially increases Tregs in patients with ACS without altering Teffs. In parallel, we used single-cell RNA-sequencing (scRNA-seq) on peripheral blood mononuclear cells (PBMCs) derived from patients in the trial to explore the mechanistic underpinnings of the potential therapeutic action of low-dose IL-2 on human immunity more broadly.

Methods

TRIAL OVERSIGHT

Low-Dose Interleukin-2 in Patients With Stable Ischemic Heart Disease and Acute Coronary Syndromes (LILACS) was an investigator-initiated, randomized, double-blind, placebo-controlled, dose-escalation clinical trial sponsored by the Cambridge University Hospitals NHS Foundation Trust, UK. The trial was approved by the UK Greater Manchester Central Research Ethics Committee and the UK Medicines and Healthcare Products Regulatory Agency. The trial protocol was published previously. The blinded trial management group met after the completion of each dose group to assess safety data before further dose escalation. After the completion of study part A, an unblinded data monitoring committee (DMC) made up of clinical researchers independent from the trial team, and not involved with trial design, assessed the unblinded safety data before progression to study part B (Supplementary Appendix, page 6).

PATIENTS

This study was conducted in two parts. Part A included patients 18 to 75 years of age who had stable ischemic heart disease, which was defined as having symptoms of angina and a coronary angiogram showing greater than 50% stenosis in at least one vessel, or patients who were more than 6 months from an ACS event regardless of their angina status. On completion of part A, part B recruited hospitalized patients 18 to 85 years age who were admitted with an acute diagnosis of either non-ST elevation MI (NSTEMI) or unstable angina. Dosing commenced within 8 days of the index admission. The full eligibility criteria are described in the Supplementary Appendix, pages 4 and 5.

TRIAL PROCEDURES

In study part A, there were five dose groups (range, 0.3 to 3.0 × 10⁶ IU daily) and participants were randomly assigned 3:2 within each group to either aldesleukin or placebo. In
study part B, two doses were explored (1.5 and 2.5 × 10^6 IU daily based on dose modeling; Supplementary Appendix, page 6), and participants were randomly assigned 3:1 within each dose group to either aldesleukin or placebo (Fig. S1). Investigators and participants were blinded to the allocation. Participants received aldesleukin (Proleukin; Novartis) or placebo over 5 consecutive days (visits V2 to V6) with a single daily, subcutaneous injection in the abdominal area. Follow-up occurred the day after the final dose (V7) and 7 days later (−3/+11-day window; V8) (Fig. S1).

END POINTS
For part A, the primary outcome was safety; in part B, the coprimary endpoints were safety and estimating the dose of aldesleukin to increase Tregs by 75% from baseline to follow-up (V7). For both study phases, safety was assessed by an open query for adverse events (AEs), a physical examination, a review of concomitant medications, vital signs, and safety blood tests (Supplementary Appendix, page 6) performed at all visits. Additionally, electrocardiograms (predose and 15, 30, and 60 minutes postdose) and cardiac telemetry were performed at dosing visits, and echocardiography was performed at screening and on final follow-up.

In part B, the 75% threshold and patient population was chosen based on observational data that showed up to a 40% reduction in Tregs in patients with acute NSTEMI and that a similar reduction of Tregs was associated with an increased incidence of MI in a population study. Tregs were defined as CD3^+CD4^+CD25^{high}CD127^{low} and expressed as the percentage of total CD4^+ T cells in peripheral blood.

Additional information on prespecified exploratory end points, including changes in lymphocyte subsets, cardiac and inflammatory biomarkers, and scRNA-seq of PBMCs can be found in the Supplementary Appendix, pages 7 to 11, and page 31.

STATISTICAL ANALYSIS
We analyzed the primary safety end point using all participants who were randomly assigned and given at least one dose of aldesleukin or placebo. The dose response was modeled using either quadratic or locally weighted regression fit graphs where the line of best fit is presented with 95% confidence intervals (95% CIs). In part B, we used a linear dose-response model to estimate the dose required to increase Tregs by 75% and the change per unit increase of aldesleukin on the Treg percentage. No multiplicity adjustments were made and the 95% CIs should not be used for inferences. The scRNA-seq statistical analysis is described in the Supplementary Appendix, pages 9 and 10.

Results
Between May 2017 and February 2019, patients at Cambridge University Hospital and Royal Papworth Hospital were recruited for this study (Fig. 1). Forty-four patients were randomly assigned to a treatment group; 3 patients withdrew before dosing and 41 patients completed the dosing protocol and follow-up. Baseline demographics are shown in Table 1.

PART A

Safety
A total of 13 AEs were reported from 8 of the 10 patients treated with placebo, and 105 AEs were reported from all 15 patients treated with aldesleukin (Tables 2 and S1). There were no serious AEs (SAEs) reported. The most common AEs were administration-site reactions (69 AEs in 16 patients), comprising injection-site erythema (86%), nodules (7%), bruising (4%), or pruritus (3%). The second most common AE was a flu-like syndrome.

Effect of Aldesleukin on Tregs and Teffs
Treatment with aldesleukin increased plasma levels of IL-2 (Fig. S2) and increased the Treg percentage (Fig. 2A) and absolute count after 5 days of treatment in a dose-dependent manner (Fig. S3). The increase in Tregs was not accompanied by an increase in Teffs (Figs. 2B and S3).

Effect of Aldesleukin on Exploratory End Points
Aldesleukin was associated with a decrease (from baseline to V7) in CD4^+ central memory T (Tcm) cells, CD8^+ T cells, and B cells in a dose-dependent manner. At the same time, there was an increase in circulating eosinophils; the highest dose (3.0 × 10^6 IU) was associated with an increase in natural killer (NK) cells and monocytes (Figs. S4 and S5). Serum analysis showed transient increases in serum IL-6 levels with an accompanying transient increase in highsensitivity C-reactive protein (hsCRP) with no effect on levels of troponin I or brain natriuretic peptide (BNP) (Figs. S6 and S7). Aldesleukin decreased high-density lipoprotein cholesterol (HDL-C) and total cholesterol in a dose-dependent
manner. Low-density lipoprotein cholesterol, triglycerides, and non–HDL-C remained stable (Fig. S8).

**PART B**

**Safety**

There were 6 AEs reported from all 4 patients treated with placebo, 22 AEs reported from all 6 patients treated with \(1.5 \times 10^6\) IU of aldesleukin daily, and 51 AEs reported from all 6 patients treated with \(2.5 \times 10^6\) IU of aldesleukin daily (Tables 2 and S1). Two SAEs were reported for two patients. One SAE occurred before dosing started and the patient was withdrawn from the study. The second SAE was in a patient admitted with a NSTEMI. Diagnostic angiography showed severe triple-vessel coronary artery disease, and the patient was awaiting inpatient coronary artery bypass surgery when they were enrolled in this trial. Dosing was completed before the patient’s scheduled surgery. On the last day of dosing, the patient developed an episode of chest pain with an associated increase in serum levels of troponin I to a peak of 2,158 ng/L (reference, 0 to 56 ng/L). The patient underwent the planned surgery and immediate postoperative recovery was uneventful. After discharge, the patient developed symptoms of a cough productive of green sputum and received treatment with a course of oral

---

Figure 1. Trial Profile.

Study part A included patients who had stable ischemic heart disease. Study part B included patients who were hospitalized with either non-ST elevation myocardial infarction or unstable angina. All patients completed dosing and follow-up. *One patient from part B group 2 had their final follow-up outside the specified time window owing to clinical reasons. IL denotes interleukin and SAE serious adverse event.
amoxicillin from their primary care physician. A second course of oral doxycycline was given on completion of the first course as a result of ongoing symptoms. A sputum culture showed no growth. On the patient’s final trial follow-up, their symptoms had resolved and their white blood count, CRP, and chest radiograph were normal.

The most common AEs were administration-site reactions (38 of 79 AEs). There were no infection-related AEs in the placebo and 1.5 × 10^6 IU dose groups. However, there were four infection-related AEs in three of six patients in the 2.5 × 10^6 IU dose group, all graded mild in severity. These AEs included two respiratory infections (in the one patient described earlier), one skin candida infection, and one arm puncture-site hematoma infection (nontrial procedural related). At the end of the trial, all data were reviewed by the independent DMC, which did not identify safety concerns in the trial that would preclude further study.

Effect of Aldesleukin on Tregs and Teffs

The percentage change of Tregs from baseline to V7 was increased for both doses tested compared with placebo (median increase [95% CI]: placebo, 6.4% [7.7 to 17.8%]; 1.5 × 10^6 IU, 95.0% [19.7 to 122.8%]; and 2.5 × 10^6 IU, 109.4% [61.8 to 173.4%]) (Fig. 2C). Regression modeling showed that the dose required to increase Tregs by 75% was 1.46 × 10^6 IU daily (95% CI, 1.06 to 1.87) (Fig. S9I). Comparing baseline to V8, a sustained increase of Tregs was seen only for the 1.5 × 10^6 IU dose (Fig. 2E and 2F). There appeared to be increased Teffs at V8 for the 2.5 × 10^6 IU dose (Figs. 2F and S9). Both doses of aldesleukin resulted in similar levels of IL-2 (area under the curve analysis - Fig. S2).

Effect of Aldesleukin on Exploratory End Points

Compared with placebo, aldesleukin resulted in a dose-dependent decrease in B cells at V7, which rebounded
Any infection included lower respiratory tract infection, hematoma infection, urinary tract infection, common cold, and skin candida infection.

Administration-site reactions included injection-site erythema, nodules, bruising, and pruritus.

Treatments administered is the total number of injections that occurred in each group. Each patient received a daily injection of IL-2/placebo for 5 consecutive days.

AE denotes adverse event, IL interleukin, and SAE serious adverse event.

![Table 2. Adverse Events for Dosed Patients.](image_url)

by V8 (Fig. S10). NK cells increased from baseline to V8 at the 2.5 × 10^6 IU dose (Fig. 2E and 2F). Aldesleukin increased eosinophil counts and transiently expanded nonclassical and intermediate monocytes (Fig. S11). There was a transient dose-dependent increase in IL-6, hsCRP, type 1 (inflammatory) cytokines, and type 2 (anti-inflammatory) cytokine and chemokine responses (Figs. S12–S14). Troponin I and BNP levels were not affected by aldesleukin (Fig. S15).

**Single-Cell Sequencing Results**

scRNA-seq and T-cell receptor (TCR) sequencing on part B PBMC samples (baseline and V7) revealed 30 cell types (Fig. S16A–S16C). Differential abundance testing using a linear mixed-effect model to account for placebo responses, age, sex, and peak serum troponin levels demonstrated that both aldesleukin doses resulted in an enrichment of Tregs, CD16^- NK cells, and CIQ-expressing nonclassical monocytes, with a decrease in CD8^+ T effector memory (Tem) cells (Fig. 3A). Data on the comparative increase in CD4^+ Tcm cells in the 2.5 × 10^6 IU group versus the 1.5 × 10^6 IU group are shown in Figure S16D.

Ligand-receptor expression was used to investigate the predicted interactions of Tregs, with a focus on interactions with antigen-presenting dendritic cells (DCs). Aldesleukin treatment most notably increased CD28–CD86 and decreased inhibitory CD52-sialic acid binding immunoglobulin-like lectin 10-mediated interactions (Fig. S17A). In the 2.5 × 10^6 IU group, there was enhanced upregulation of some inhibitory interactions, including via FAS (CD95)–Tumor Necrosis Factor Superfamily Member 13 (TNFSF13) (proapoptotic activity), Human Leukocyte Antigen (HLA)-F–Leukocyte Ig-like Receptor (LILR) B1 (LILRB) proteins contain immunoreceptor tyrosine-based inhibitory motif (ITIM) domains, and the emergence of a potential tissue recruitment signal via upregulation of Signal Regulatory Protein Gamma (SIRPG) (Fig. S17A).

Analysis of TCRs enables an assessment of whether clones of T cells recognizing antigens via the same TCR were present after MI and how this was affected by aldesleukin. Clonotype expansion in untreated patients was largely restricted to the central memory compartment, and this was diminished in both the 1.5 and 2.5 × 10^6 IU treatment groups (Fig. 3B). There was a modest increase in larger clones within the CD4^+ Tregs after 1.5 × 10^6 IU dose treatment compared with naive cells, but this effect was more prominent in patients treated with 2.5 × 10^6 IU. However, there was also clonal expansion in the CD4^+ effector memory compartment at the higher dose (Fig. 3E).
In both the 1.5 and 2.5 × 10^6 IU dose groups, gene set enrichment analysis showed an increase in several gene sets related to metabolism in CD4^+ Tregs, including Mechanistic Target of Rapamycin Complex (MTORC) signaling, hypoxia, and oxidative phosphorylation (notable given the effects of cell metabolism on T-cell differentiation and function\textsuperscript{21}), as well in IL-2-Signal Transducer and Activator of Transcription (STAT) 5 signaling pathway genes (Fig. 3C). In contrast, enrichment of these pathways in CD4^+ Tem and CD4^+ Tcm cells was only observed after the 2.5 × 10^6 IU dose (Fig. 3C). The most upregulated pathway after 1.5 × 10^6 IU treatment in Tregs was epithelial-mesenchymal transition, including, for example, IL-32 and Transforming Growth Factor Beta 1 (TGFβ1), with the latter being a major immunoregulatory cytokine and upregulated in the 1.5 × 10^6 IU group alone (Fig. S17B). Selected metabolic pathway genes were validated in ex vivo stimulated human CD4^+ Tregs (Fig. S17C), and ex vivo Seahorse analysis confirmed increases in extracellular acidification (a readout of glycolysis), the oxygen consumption rate (a readout of oxidative phosphorylation), and production of mitochondrial reactive oxygen species in the presence of IL-2 (Fig. S17D).

**Discussion**

Our data show that the administration of IL-2 (aldesleukin) in a small number of patients with stable ischemic heart disease and ACS — conditions in which its use is currently contraindicated — was not associated with widespread SAEs. The majority of AEs were self-limiting side effects, which were largely, but not completely, of mild intensity. The two most common AEs, administration-site reactions and a flu-like syndrome, are both known side effects of aldesleukin.
Figure 3. Single-Cell RNA-Sequencing.

(A) Differential abundance testing of single-cell neighborhoods between untreated and post–interleukin (IL)-2 treatment groups with a negative binomial generalized linear mixed-effects model (n = 20 samples from 10 patients, 4 treated with placebo and 6 with IL-2). The plot shows the beta coefficients for each neighborhood assigned to corresponding cell types where positive and negative coefficient values are interpreted as enriched or depleted after IL-2 treatment, respectively. Differentially abundant neighborhoods are colored according to the beta coefficient value from blue to white to red, where white indicates a value of 0 (no change). Nonsignificant neighborhoods are colored gray. (B) T-cell receptor (TCR) clonotype size for CD4 T cells visualized on single-cell UMAP (Uniform Manifold Approximation and Projection) for each treatment group. TCR clonotypes were defined based on the identical CDR3 amino acid sequence of TCR-alpha-beta pairs between cells. Clonotype sizes are colored with increasing values corresponding to gradients from white to blue for non-regulatory T cells (Tregs) and from white to red for Tregs. The maximum clonotype size is capped at 5. In A–C, the untreated group includes all samples from the placebo group (pre and post) and pretreatment samples from the IL-2 dosage groups. (C) Gene set enrichment analysis (GSEA) of CD4 central memory T (Tcm) cells, effector memory T (Tem) cells, and Tregs for untreated versus post–IL-2 treatment groups. The size of the circles indicates the (absolute) normalized enrichment score (NES). A GSEA (permutation) nominal p-value (pval) < 0.05 and a false discrimination rate (FDR) < 0.25 are considered statistically significant. Blue, $1.5 \times 10^6$ IU (1.5MIU) versus untreated; orange, $2.5 \times 10^6$ IU (2.5MIU) versus untreated; and gray, not significant. NK denotes natural killer.
The safety profile we observed in our early-phase trial was similar to other studies using low-dose IL-2 in autoimmune disease. There was a higher rate of infections (three of six patients) at the 2.5 × 10^6 IU dose in study part B, which was not observed in study part A at the 2.4 or 3 × 10^6 IU dose. Although the number of patients within each dose group was small, this safety signal will require further evaluation in a larger trial. Although infections were not observed in other trials at similar or higher doses of IL-2 despite concomitant immunosuppression, these trials did not include patients with ACS.

The estimated dose of aldesleukin to increase Tregs by 75% in patients with ACS was 1.5 × 10^6 IU daily. This dose is further supported by the more sustained increase in the percentage of Tregs at the later V8 time point without increases in T eff or NK cells associated with the higher 2.5 × 10^6 IU dose. Using scRNA-seq, we observed an expansion of Tregs with both doses; in addition, we showed that the 2.5 × 10^6 IU dose also increased CD4^+ Tcm cells and was associated with clonal expansion in the CD4^+ Tem compartment, representing cells with the potential to increase inflammation after an MI. The ligand-receptor expression analysis offered insight into the possible mechanisms of aldesleukin. The observed CD28–CD86 interaction is an important activating costimulatory signal by which DCs might cause Treg expansion. We further speculate that decreases in CD52-SIGLEC10-mediated interactions will likely promote Treg expansion and activation, whereas an increase in SIRPG is known to promote T-cell transendothelial migration into tissue.

It is increasingly appreciated that the functional profile of Tregs is dependent on their metabolic state, which we explored using scRNA-seq and confirmed using ex vivo Seahorse analysis. The 2.5 × 10^6 IU dose was associated with an increase in Treg glycolysis, which is known to promote cell growth and migration at the cost of immunosuppressive function. In contrast, the lower 1.5 × 10^6 IU dose was able to more selectively increase oxidative phosphorylation, which has been linked with increased suppressive function. In addition, the 2.5 × 10^6 IU dose increased several metabolic pathways associated with activation and proliferation in CD4^+ Tem and Tcm cells. Taken together, these results show that although both doses induce CD4^+ Treg activation and expansion, the 1.5 × 10^6 IU dose may have a more beneficial clinical effect.

Aldesleukin led to dose-dependent decreases in B cells and CD8^+ T cells. The mechanism for this change needs further exploration. In the heightened inflammatory state after an MI, preclinical models show that CD8^+ T cells and B cells have a detrimental effect on both atherosclerosis and remodeling; therefore, their reduction may represent an additional mechanism of benefit of low-dose aldesleukin. At the higher doses, increases in eosinophils were observed, which may contribute to myocardial repair after ischemic injury.

We acknowledge several limitations of our trial design. Early-phase clinical trials, by nature, are small and therefore only provide provisional data on safety. In this trial, we treated patients for 5 days; however, larger and longer trials are underway (ClinicalTrials.gov NCT04241601) in a broader population of patients with ACS. Although we report gene expression profile changes that are consistent with increased suppressive function, Treg function was not assessed directly. However, previous evidence shows that low-dose IL-2 increased not only the Treg number but also suppressive function.

The scRNA-seq data are insightful; their link to clinical outcomes is an unexplored field but has the potential to identify valuable biomarkers and to reveal unappreciated mechanisms of drug activity.

In summary, we present novel safety and mechanistic data for the use of low-dose IL-2 for patients with ACS as a strategy to increase Tregs. We have calculated a dose for therapeutic use in this patient group, which has already been taken into a phase 2b study (ClinicalTrials.gov NCT04241601). The data from this study provide important insights into the biology and utility of IL-2 for patients with ACS.

Funded by grants from the Medical Research Council (MRC; MR/N028015/1), the British Heart Foundation Cambridge Centre of Excellence (RE/18/1/34212), and the National Institute for Health Research (NIHR) Cambridge Biomedical Research Centre (BRC-1215-20014). The Wellcome Trust–MRC Cambridge Stem Cell Institute is supported by a grant from the Wellcome Trust (203151/Z/16/Z). We acknowledge Cambridge University Hospitals as a sponsor of this trial. The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

Disclosure
Author disclosures and other supplementary materials are available at evidence.nejm.org.
A data sharing statement provided by the authors is available at evidence.nejm.org.

We thank the patients for contributing to this trial. We acknowledge the Department of Clinical Immunology and the Core Biochemistry Assay Laboratory at Cambridge University Hospitals NHS Foundation Trust for support with sample analysis. We also acknowledge the members of the independent data monitoring committee for their oversight during this trial: Professor Tim Mant (Chair), Dr. Kevin O’Shaughnessy, and Professor Robin Choudhury. Finally, we acknowledge staff and facility support from the Cambridge Clinical Research Centre and Cambridge Clinical Trials Unit. For the purpose of Open Access, the author has applied a CC BY public copyright license to any Author Accepted Manuscript version arising from this submission.

Author Affiliations
1 Division of Cardiovascular Medicine, Department of Medicine, University of Cambridge, Cambridge, United Kingdom
2 Molecular Immunity Unit, Department of Medicine, University of Cambridge, Cambridge, United Kingdom
3 Cellular Genomics, Wellcome Sanger Institute, Hinxton, United Kingdom
4 Division of Experimental Medicine and Immunotherapeutics, Department of Medicine, University of Cambridge, Cambridge, United Kingdom
5 Department of Inflammation, Immunopathology, and Biotherapy, Pitie-Salpetriere Hospital, Assistance Publique-Hopitaux de Paris, Paris, France
6 Paris Cardiovascular Research Center, Universite de Paris, Institut National de la Santé et de la Recherche Medicale, Paris, France
7 Department of Haematology, University of Cambridge, Cambridge, United Kingdom
8 Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, Cambridge, United Kingdom
9 Department of Cardiology, Royal Papworth Hospital NHS Foundation Trust, Cambridge, United Kingdom
10 Cambridge Clinical Trials Unit, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom
11 Cambridge Institute for Therapeutic Immunology and Infectious Disease, Cambridge Biomedical Campus, Cambridge, United Kingdom

Author disclosures and other supplementary material are available with the full text of this article at evidence.nejm.org.

References


