Effect of interleukin-6 receptor blockade on surrogates of vascular risk in rheumatoid arthritis: MEASURE, a randomised, placebo-controlled study

Iain B McInnes,1 Liz Thompson,2 Jon T Giles,3 Joan M Bathon,4 Jane E Salmon,4 Andre D Beaulieu,5 Christine E Codding,6 Timothy H Carlson,7 Christian Delles,1 Janet S Lee,8 Naveed Sattar1

ABSTRACT

Objectives The interleukin-6 receptor (IL-6R) blocker tocilizumab (TCZ) reduces inflammatory disease activity in rheumatoid arthritis (RA) but elevates lipid concentrations in some patients. We aimed to characterise the impact of IL-6R inhibition on established and novel risk factors in active RA.

Methods Randomised, multicentre, two-part, phase III trial (24-week double-blind, 80-week open-label), MEASURE, evaluated lipid and lipoprotein levels, high-density lipoprotein (HDL) particle composition, markers of coagulation, thrombosis and vascular function by pulse wave velocity (PWV) in 132 patients with RA who received TCZ or placebo.

Results Median total-cholesterol, low-density lipoprotein-cholesterol (LDL-C) and triglyceride levels increased in TCZ versus placebo recipients by week 12 (12.6% vs 1.7%, 28.1% vs 2.2%, 10.6% vs −1.9%, respectively; all p<0.01). There were no significant differences in mean small LDL, mean oxidised LDL or total HDL-C concentrations. However, HDL-associated serum amyloid A content decreased in TCZ recipients. TCZ also induced reductions (>30%) in secretory phospholipase A2-IIA, lipoprotein(a), fibrinogen and D-dimers and elevation of paraoxonase (all p<0.0001 vs placebo). The ApoB/ApoA1 ratio remained stable over time in both groups. PWV decreases were greater with placebo than TCZ at 12 weeks (adjusted mean difference 0.79 m/s (95% CI 0.22 to 1.35; p=0.0067)).

Conclusions These data provide the first detailed evidence for the modulation of lipoprotein particles and other surrogates of vascular risk with IL-6R inhibition. When compared with placebo, TCZ induced elevations in LDL-C but altered HDL particles towards an anti-inflammatory composition and favourably modified most, but not all, measured vascular risk surrogates. The net effect of such changes for cardiovascular risk requires determination.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with clinically important comorbidities, including accelerated cardiovascular risk.1 The latter is not explained by conventional risk factors (eg, hypertension, obesity), suggesting that additional pathways contribute to adverse outcomes. These may reflect common genetic or environmental aetiological factors or the impact of chronic inflammation on underlying atherosclerotic disease burden, operating through circulating cytokines, immune complexes, complement factors and acute-phase reactants.2–4 Furthermore, it is recognised that absolute circulating lipid concentrations are modified in RA, likely reflecting regulatory integration of metabolic and inflammatory molecular networks.5 In general, high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) levels are reduced in active disease6 and may increase on the initiation of effective therapeutics regardless of modality.7 Moreover, interpretation of lipid particle concentrations may be further complicated by changes in size and composition associated with inflammation. For example, small LDL-C particles may confer more atherogenic risk than larger LDL-C particles.8 In inflammatory conditions, HDL particles are associated with increased serum amyloid A (SAA) content, representing a potentially proatherogenic phenotype.9 The impact of therapy on subparticle components in RA has not been well characterised. Similarly, the effect of therapy on other lipid particles causally associated with vascular disease, such as lipoprotein(a) (Lp [a]),10 and on clotting factors, such as fibrinogen or markers of activated clotting such as D-dimer,11 is poorly understood.

Interleukin-6 (IL-6) plays an important role in various inflammatory effector pathways in RA through B-cell, fibroblast and osteoclast activation. Additionally, it mediates systemic manifestations of disease operating through hepatic and central neuro-immune pathways.12 Intriguingly, elevated IL-6 levels are independently associated with increased cardiovascular risk, including fatal myocardial infarction and cerebrovascular accident, in the general population.13 14 The mechanisms mediating such epidemiological observations are poorly understood but are likely to be commensurate with the fundamental role played by inflammatory pathways in the pathogenesis of atherosclerosis, the systemic functional activities of IL-6 conferred by widespread gp130 receptor membrane expression and the existence of soluble IL-6 receptor (IL-6R).15 Moreover, loss-of-function IL-6R polymorphisms are associated with reduced vascular risk.16 17

Tocilizumab (TCZ) is a monoclonal antibody targeting IL-6R (membrane-bound and soluble) that reduces inflammation and articular damage in patients with RA. In phase II and III trials, moderate elevations of LDL-C, HDL-C and triglycerides were apparent in RA patients treated with TCZ.7 The
atherogenic implications of these changes are unknown. Similarly, the effect of IL-6R blockade on vascular physiology parameters (eg, as assessed by pulse wave velocity (PWV)) has been minimally explored. PWV is a measure of early structural vascular changes and has been shown to respond within 3 months to changes in vascular inflammation. Thus, given its mode of action, TCZ provides a highly specific molecular intervention with which to dissect the role of IL-6 in the modulation of lipid particles and the regulation of other vascular risk factors in patients with chronic inflammation. We report herein the results of a placebo-controlled trial that sought to define the effects of TCZ on a range of vascular risk surrogates in patients with RA. Our primary hypotheses were that PWV and small LDL particles would be significantly reduced by TCZ.

METHODS
Patients
This trial, conducted independently of the pivotal RA trials, was approved by an independent ethics committee or institutional review board, and all patients gave written informed consent for participation in the trial. Adult patients with moderately to severely active RA (diagnosed per American College of Rheumatology (ACR) criteria) of more than 6 months’ duration were recruited. Enrolment criteria included inadequate response to stable methotrexate (MTX) therapy, exemplified by a swollen joint count (SJC) ≥6 and a tender joint count (TJC) ≥6, together with C-reactive protein (CRP) >10 mg/L or erythrocyte sedimentation rate (ESR) >28 mm/h. Patients with inadequate response to an antitumour necrosis factor-α (αTNF) agent during the 6 months before baseline or to more than two previous αTNF agents were ineligible. MTX therapy was continued during the study. Initiation of lipid-lowering, oral antidiabetic or antihypertensive medications or change in dose within 12 weeks of baseline was prohibited, and glucocorticoid doses (≤10 mg) had to remain stable. Patients were stratified at randomisation by age (<52 vs ≥52 years), mean arterial blood pressure (<93.3 vs ≥93.3 mm Hg) and CRP (<1.66 vs ≥1.66 mg/dL).

 Procedures
This two-arm, randomised, multicentre, double-blind, placebo-controlled, parallel-group, phase III study was conducted in the USA, Canada and the UK (figure 1) at 34 sites (the MEASURE study). Patients were randomly assigned using an interactive voice response system to blinded (patient and treating clinical assessor, and certification was provided by AtCor. All PWV scans were reviewed by AtCor, and only those meeting predetermined quality control measures were accepted for analysis (details in online supplementary material).

Lipid and biomarker assays
Commercial assays were used to measure cholesterol, triglycerides, apolipoproteins A1 and B (ApoA1 and ApoB), CRP (by high-sensitivity assay) and ESR (all assays conducted or facilitated by Covance Laboratories, Greenfield, Indiana, USA). Serum lipid subclasses were characterised by nuclear magnetic resonance (NMR; Liposcience, Raleigh, North Carolina, USA). Assays for HDL-associated SAA and serum paraoxonase, secretory phospholipase A2-IA (sPLA2-IA), oxidised LDL, d-dimer, fibrinogen, Lp(a) and haptoglobin were performed at Pacific Biomarkers, Inc. (Seattle, Washington, USA). Specific details are provided as online supplementary material.

Vascular physiology
Arterial stiffness was assessed by PWV according to the manufacturer’s instructions using a pulse wave analysis apparatus (Sphygmocor; AtCor, San Jose, California, USA). Blinded assessors from all centres underwent training with an expert assessor, and certification was provided by AtCor. All PWV assessments were performed at 12 weeks using last-observation-carried-forward to impute missing data at the analysis time point. Only measurements recorded before escape therapy were carried forward. All other exploratory end points were summarised for the ITT population observed cases, without imputation of missing data and excluding escape data. All laboratory parameter values were converted to SI units; for lipid parameters, only the latest fasted values within the time window were included. Assumptions of normality and homogeneity of the variance were assessed by inspecting normal probability plots, plots of standardised residual versus predicted values and plots of standardised residual versus continuous covariates. Primary end points in the study were analysed based on a normal distribution; however, because baseline values for several laboratory assessments in this study demonstrated non-normal distributions, values for exploratory serum analytes are presented as medians or median percent change from baseline. Exploratory analyses, based on observed cases, were performed using the non-parametric Kruskal–Wallis test. Hodges–Lehmann estimates of location shift and 95% CIs are presented. No adjustments for multiplicity were performed.

RESULTS
Sixty-nine patients were randomly assigned to receive TCZ +MTX and sixty-three to receive placebo+MTX (figure 1). Sixty-five and sixty patients, respectively, completed 12 weeks of therapy. One patient in each arm withdrew because of an adverse event; one patient on placebo withdrew because of insufficient

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therapeutic response, three patients in the TCZ arm refused treatment and one patient in the TCZ arm was withdrawn because of a protocol violation. Two patients in the TCZ arm had their lipid-lowering medications changed; though included in the ITT analyses, they were excluded from the per-protocol analyses. Of 124 patients who completed blinded treatment, 117 elected to continue to the open-label phase; 92 completed treatment for 96 weeks. Demographic and baseline disease characteristics (table 1) were similar to those of the TCZ phase III cohort.20

Moreover, 30% and 39% of the patients in the placebo and TCZ arms, respectively, had previously received aTNFs. TCZ efficacy, assessed by change in disease activity score at 28 joints (DAS28) at weeks 12 and 24 or by ACR 20/50/70 proportional changes at week 24, was similar to that previously observed and differed significantly from that of placebo (see online supplementary figure S1). Adverse events and serious adverse events occurring during the trial were similar to those observed in previous TCZ studies (see online supplementary table S1).

Figure 1  Study design. MTX, methotrexate; TCZ, tocilizumab. (A) *Patients who did not achieve ≥20% improvement from baseline in swollen and tender joint counts at week 16 were offered escape therapy with open-label TCZ 8 mg/kg. †60 placebo+MTX and 65 TCZ+MTX patients completed 12 weeks. §59 placebo+MTX and 65 TCZ+MTX patients completed 24 weeks. (B) *TCZ 8 mg/kg every 4 weeks+background MTX (7.5–25 mg weekly). †Escape therapy, open-label TCZ (8 mg/kg every 4 weeks+background MTX). ‡Patients who received at least one dose of TCZ (double-blind or open-label).
Consistent with results from TCZ phase III clinical trials, median total cholesterol and LDL-C levels increased in TCZ recipients but did not change in placebo patients (table 2). In contrast, no statistically significant difference in the co-primary outcome of concentration of small LDL particles was observed at either week 12 (adjusted mean difference −0.99 vs −0.21 m/s; p=0.30) (table 3). Technical challenges across sites were recorded in a substantial number of case report forms. There was no evidence of change in blood pressure by treatment arm before or after infusions (data available on request). Ten (17.5%) patients who received placebo and nine (15.5%) who received TCZ did not meet the quality control standards established for all measured PWV parameters. No statistically significant changes occurred in total serum HDL-C levels in the study. NMR evaluation of HDL, however, revealed differential effects of TCZ versus placebo across particle

### Table 1 Demographic and disease factors at baseline

| Placebo+MTX (n=63)* | TCZ+MTX (n=69) |
|---------------------|-----------------|
| **Female, n (%)**   | 47 (75)         | 57 (83)         |
| Age, years          | 57.0 (50.0−64.0) | 57.0 (49.0−62.0) |
| Weight, kg          | 82.0 (65.0−92.1) | 77.4 (67.0−86.5) |
| BMI, kg/m², median (range) | 29.2 (18.3−49.6) | 29.2 (19.4−57.3) |
| Current smoker, n (%) | 14 (22)     | 19 (28)         |
| History of diabetes, n (%) | 4 (6)       | 6 (9)           |
| Duration of RA, years | 6.8 (2.4−9.9)  | 7.0 (2.0−16.2)  |
| DAS28                | 6.6 (5.8−7.3)   | 6.8 (5.9−7.4)   |
| CRP, mg/dL           | 0.88 (0.39−1.86)| 0.94 (0.52−2.65) |
| Statin use, n (%)    | 10 (16)        | 10 (14)         |
| Previous aTNF, n (%) | 19 (30)       | 27 (39)         |
| Oral steroid use, n (%) | 17 (27)     | 20 (29)         |
| Baseline MTX dose, mg/week | 15.0 (15.0−20.0)| 15.0 (15.0−20.0)|

*One patient randomly assigned to placebo+MTX actually received one dose of TCZ and was therefore included in the TCZ group for the safety analyses.

Data are presented as median (IQR) unless otherwise indicated.

### Table 2 Percentage change from baseline to week 12 in lipid parameters and lipid particles* (observed cases), ITT population

| Placebo+MTX (n=63) | TCZ 8 mg/kg+MTX (n=69) |
|---------------------|------------------------|
| **Total cholesterol (mmol/L)**  | 4.8  | 4.3−5.5  |
| Baseline             | 55   | 55      |
| Week 12              | 60   | 60      |
| **LDL-C (mmol/L)**   | 3.1  | 2.5−3.5  |
| Baseline             | 55   | 55      |
| Week 12              | 56   | 56      |
| **HDL-C (mmol/L)**   | 1.3  | 1.1−1.6  |
| Baseline             | 58   | 54      |
| Week 12              | 60   | 60      |
| **Triglycerides (mmol/L)** | 1.3  | 1.1−1.9  |
| Baseline             | 58   | 55      |
| Week 12              | 60   | 60      |
| **Total cholesterol/HDL ratio** | 3.8  | 3.2−4.4  |
| Baseline             | 55   | 55      |
| Week 12              | 60   | 60      |
| **ApoB/ApoA1 (ratio)** | 0.67 | 0.57−0.77 |
| Baseline             | 55   | 55      |
| Week 12              | 60   | 60      |
| **Large VLDL/chylomicrons (nmol/L)** | 1.1  | 0.5−2.8  |
| Baseline             | 59   | 53      |
| Week 12              | 60   | 60      |

*One patient randomly assigned to placebo+MTX actually received one dose of TCZ and was therefore included in the TCZ group for the safety analyses.

Data are presented as median (IQR) unless otherwise indicated.

**Placebo+MTX**

- Median IQR n
- Actual values Change from baseline (%) Actual values Change from baseline (%)

**TCZ+MTX**

- Median IQR n
- Actual values Change from baseline (%) Actual values Change from baseline (%)

**Estimate** (95% CI)

**p for difference at week 12**

**p=0.0004**

**p=0.0076**

**p=0.0011**

**p=0.0008**

**p=0.0001**
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Table 2 Continued

| Placebo+MTX (n=63) | TCZ 8 mg/kg+MTX (n=69) |
|---------------------|-------------------------|
| Actual values | Change from baseline (%) | Actual values | Change from baseline (%) | Estimate* (95% CI) |
| | n Median IQR | n Median IQR | n Median IQR | n Median IQR | p for difference at week 12 |
| Medium VLDL/chylomicrons (nmol/L) | | | | | |
| Baseline | 59 22.1 12.0–38.3 | 56 – – | 63 22.0 11.0–32.7 | 55 – – | p=0.0008 |
| Week 12 | 60 21.9 12.1–30.5 | 56 – – | 59 28.2 17.2–47.4 | 55 57.7 –19.1–123.6 | 41.3 (9.2 to 77.5) |
| Small VLDL/chylomicrons (nmol/L) | | | | | |
| Baseline | 59 35.6 29.1–48.1 | 56 – – | 63 30.4 18.7–40.0 | 55 – – | p=0.0001 |
| Week 12 | 60 36.2 26.2–46.2 | 56 – – | 59 40.1 26.5–57.2 | 55 31.4 10.2–91.5 | 42.3 (24.1 to 60.5) |
| LDL particles (nmol/L) | | | | | |
| Baseline | 59 33.0 9.0–57.0 | 51 – – | 63 31.0 7.0–71.0 | 46 – – | p=0.0012 |
| Week 12 | 60 36.0 9.0–59.5 | 51 – – | 59 39.0 11.0–92.0 | 46 33.5 –60.9–222.6 | 14.3 (–37.3 to 70.0) |
| Large LDL particles (nmol/L) | | | | | |
| Baseline | 59 396.0 261.0–508.0 | 56 – – | 63 405.0 253.0–515.0 | 55 – – | p=0.0053 |
| Week 12 | 60 479.5 331.5–544.5 | 56 13.2 –9.4–35.3 | 59 495.0 306.0–605.0 | 55 18.6 –18.6–46.9 | –0.16 (–17.9 to 17.2) |
| Medium HDL particles (μmol/L) | | | | | |
| Baseline | 59 6.9 4.7–9.9 | 56 – – | 63 7.0 4.6–10.6 | 55 – – | p=0.4140 |
| Week 12 | 60 7.8 5.5–11.0 | 56 1.8 –15.5–13.4 | 59 8.4 5.3–11.6 | 55 5.6 –8.6–23.7 | 4.8 (–5.9 to 15.1) |
| Small HDL particles (μmol/L) | | | | | |
| Baseline | 59 3.0 0.5–6.4 | 46 – – | 63 4.8 1.9–7.6 | 52 – – | p=0.0454 |
| Week 12 | 60 3.2 1.5–7.4 | 46 2.0 –30.4–56.5 | 59 4.2 1.2–7.5 | 52 –25.0 –71.5–25.8 | –30.7 (–61.3 to –0.23) |
| Data were missing at time points (including baseline) for some parameters. All values have been converted to SI units. No imputation was used for missing values. Only the latest fasted values within the time window are included.
* Hodges–Lehmann estimate of location shift (pseudo-median). p was calculated from Kruskal–Wallis test.
†Percentage change from baseline values includes only patients with both baseline and 12-week values.
Apo, apolipoprotein; C, cholesterol; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; ITT, intent-to-treat; LDL, low-density lipoprotein; MTX, methotrexate; TCZ, tocilizumab; VLDL, very low-density lipoprotein.

sizes, with observed elevations in small HDL concentration and reductions in medium HDL (table 2). A significant reduction (by 78%) was observed in the TCZ arm for HDL-associated SAA. In contrast, a significant increase (by 16%) was noted in the antioxidant enzyme paraoxonase I, which is almost exclusively carried in serum HDL (figure 2D–F). sPLA2-IIA was reduced (by 61%) in TCZ recipients. No changes were observed in the control arm (figure 3). Similarly, rapid and sustained reductions in fibrinogen (by 47% from baseline) and D-dimer (by 62% from baseline) were observed in recipients of TCZ, whereas no changes were observed in the control arm (figure 3). All changes remained stable throughout TCZ treatment. Finally, the ApoB/ApoA1 ratio did not change throughout the study in either group (table 2).

DISCUSSION

We herein tested the primary hypotheses that TCZ in comparison to placebo would significantly lower PWV and the amount of small, dense LDL. However, neither hypothesis was supported. There was no change in the amount of small, dense LDL, and though PWV declined in both groups it did so more in placebo recipients. We also performed extensive additional analyses to investigate the wider impact of IL-6R inhibition on a range of vascular risk factors. Thus, we report that TCZ did modulate lipid particle levels (LDL, HDL, VLDL) and composition (HDL-associated SAA), together with a number of other inflammatory (CRP, paraoxonase) and vascular (Lp(a), D-dimer, fibrinogen) risk factors, suggesting potential modulation of the net atherogenic risk profile.
Consistent with results from other TCZ phase III clinical trials, total cholesterol, LDL-C and triglyceride levels increased in TCZ-treated patients but were minimally changed in placebo patients. In contrast, small LDL particle concentrations, considered proatherogenic, remained similar in TCZ-treated and placebo patients. Moreover, we observed a decrease (>30%) in Lp(a), a risk factor independently associated with vascular events. NMR assessment of lipoprotein subclasses revealed clear and sizeable changes in all classes of VLDL particles, the functional significance of which is yet unclear.

By contrast, we did not see a reduction in PWV with TCZ but rather a significant reduction in the placebo group at 12 weeks relative to TCZ recipients, though this pattern was not sustained to the 24-week measurement. This finding, therefore, contradicts our original hypothesis. It should be noted, however, that technical challenges during the conduct of this multicentre trial led to approximately 15% of the measurements being substandard. Because of the severity of disease in study participants who had reduced mobility, contractures and joint pain, the procedure proved more difficult than anticipated. Nevertheless, it appears that PWV is not necessarily reduced with TCZ, though further studies with other vascular function measures not influenced by limited mobility (eg, peripheral arterial tonometry as a measure of endothelial function) would be useful. Our findings contrast with those of Kume et al, who observed similar reductions in cardio-ankle vascular index and aortic augmentation index for patients treated with TCZ, adalimumab or etanercept. Speculatively, it remains possible that disease-related vascular changes in patients in the present study (in which mean DAS28 was high) were much more progressed (and, thus, less reversible) than in patients studied in other trials.

![Figure 2](A–F) Effects on lipoproteins (TCZ vs placebo). HDL, high-density lipoprotein; LDL, low-density lipoprotein; MTX, methotrexate; SAA, serum amyloid A; sPLA2-IIA, secretory phospholipase A2-IIA; TCZ, tocilizumab. *p<0.0001 (TCZ vs placebo).
Increases in HDL particle number, measured by NMR, occurred primarily in small particles. Our demonstration of a significant increase in the concentration of small HDL particles with TCZ treatment is consistent with a potential ‘normalisation’ of small HDL particle levels. Small HDL particle numbers, measured by NMR, in two independent studies were lower in RA patients than in controls despite similar HDL-C concentrations.\(^5\)\(^6\) Furthermore, the observed significant reduction in medium HDL and HDL-SAA concentrations, along with the increase in paraoxonase, an antioxidant enzyme associated with HDL, suggests remodelling of HDL particles from a pro-inflammatory to an anti-inflammatory phenotype in response to TCZ treatment. Overall, such changes in HDL particle composition with TCZ are consistent with the results of a recent study of aTNFs in patients with ankylosing spondylitis.\(^22\) Although it is unclear to what extent small HDL particles measured by NMR corroborate with those measured by other methods, small HDL particles may be more active in cholesterol efflux and anti-inflammatory functions,\(^23\) though this observation remains debated.

Previous studies have demonstrated lower levels of LDL-C in patients with active RA than in controls.\(^6\) Such decreases may result from increased catabolism (including by scavenger

**Table 3** Change from baseline in PWV (LOCF), ITT population

|                      | Placebo+MTX (n=63) | TCZ 8 mg/kg+MTX (n=69) | 95% CI (p) for difference |
|----------------------|--------------------|------------------------|--------------------------|
| **Baseline**         |                    |                        |                          |
| n                    | 59                 | 69                     |                          |
| Mean (SD) PWV, m/s   | 9.0 (2.5)          | 9.0 (2.0)              |                          |
| **Week 12**          |                    |                        |                          |
| n                    | 62                 | 69                     |                          |
| Mean (SD) PWV, m/s   | 8.4 (1.8)          | 8.9 (2.5)              |                          |
| Mean change from baseline in PWV, m/s | –0.99 | –0.21 | 0.22 to 1.35 (p=0.0067) |
| **Week 24**          |                    |                        |                          |
| n                    | 62                 | 69                     |                          |
| Mean (SD) PWV, m/s   | 8.9 (2.0)          | 9.0 (2.3)              |                          |
| Mean change from baseline in PWV, m/s | –0.47 | –0.17 | –0.27 to 0.87 (p=0.3042) |

LOCF was used for missing values. Only postbaseline and pre-escape therapy scores were carried forward. All assessments were set to missing from the time of escape therapy. Mean change from baseline was adjusted for baseline age, C-reactive protein level and mean arterial pressure. ITT, intent-to-treat; LOCF, last-observation-carried-forward; MTX, methotrexate; PWV, pulse wave velocity; TCZ, tocilizumab.

**Figure 3** (A–D) Effects on inflammatory and thrombotic markers (TCZ vs placebo). hs-CRP, high-sensitivity C-reactive protein; MTX, methotrexate; TCZ, tocilizumab. *p<0.0001 (TCZ vs placebo).
receptors), increased particle retention in tissue, or both, rather than from decreased lipid production. Increased cholesterol retention under conditions of elevated IL-6/IL-6R levels have been hypothesised to result from increased surface density on multiple tissues of LDL receptor, VLDL receptor and scavenger receptors, leading to excess internalisation of VLDL and LDL. Furthermore, sPLA2-IIA expression, augmented by IL-6, leads to phospholipid hydrolysis of LDL and increases LDL uptake in tissues. Consistent with our results and the TCZ phase III programme, the TCZ-based IL-6 signal inhibition may reduce various receptor surface levels and sPLA2-IIA levels, leading to both decreased LDL and VLDL tissue retention and elevated circulating levels.

Decreased thrombotic potential in TCZ-treated RA patients is indicated by declines in circulating fibrinogen and D-dimer levels. Although the reduction in fibrinogen, an acute-phase protein, with TCZ is predictable, the reduction of D-dimer is of particular interest because it represents the most widely used clinical marker of activated blood coagulation. Moreover, several prospective studies have linked elevated D-dimer levels to heightened risk for vascular events independently of established risk factors. Similarly, the sizeable reduction in Lp(a) observed with TCZ is of considerable interest because recent genetic and epidemiological evidence suggests Lp(a) is causally linked to cardiovascular events in the general population. Our Lp(a) observations also extend findings from an earlier randomised, placebo-controlled study linking aTNF blockade to dose-dependent reductions in Lp(a) in patients with psoriatic arthritis. Collectively, these changes suggest a reduction in thrombotic potential with TCZ in patients with active RA. Of course, the net effect of TCZ-induced changes to vascular outcomes can be robustly tested only in the context of vascular outcomes in prospective studies.

In summary, the results of this randomised, placebo-controlled study suggest that IL-6R blockade with TCZ in patients with active RA not only reduces markers of inflammation but also affects quantitative and qualitative changes in lipids and lipoproteins. Such changes include a global increase in LDL-C concentration, in line with findings from other biological studies, and apparently favourable changes to HDL particle composition, rendering them less pro-inflammatory. In addition, marked reductions in haemostatic and Lp(a) markers were observed, though PWV did not change favourably. Future determination of the net vascular effect of such changes in RA patients—and potentially other groups of patients—is of major interest, particularly given the recent data from large-scale (>130 000 subjects, >25 000 coronary heart disease cases) genome-wide association studies that suggest a potentially detrimental effect of IL-6R signalling on the risk for coronary heart disease.

Author affiliations
1 University of Glasgow, Glasgow, UK
2 Roche Products Ltd, Welwyn Garden City, UK
3 Columbia University, New York, New York, USA
4 Hospital for Special Surgery—Weill Cornell Medical College, New York, New York, USA
5 Centre Hospitalier de l’Université Laval, Quebec City, Quebec, Canada
6 Health Research of Oklahoma, Oklahoma City, Oklahoma, USA
7 Pacific Biomarkers, Seattle, Washington, USA
8 Roche, Nutley, New Jersey, USA

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Contributors IBM was involved in the design of the study, conducting the research, analysis and interpretation of the data, drafting of the manuscript, and approval of the final draft to be published. LT was involved in the design of the study, data collection, analysis and interpretation of the data, drafting of the manuscript and approval of the final draft to be published. JTG was involved in conducting the research, analysis and interpretation of data, drafting of the manuscript and approval of the final draft to be published. JMB and JSL were involved in the design of the study, analysis and interpretation of the data, drafting of the manuscript and approval of the final draft to be published. JES and NS were involved in the design of the study, drafting of the manuscript, and approval of the final draft to be published. ADB was involved in conducting the research, drafting of the manuscript and approval of the final draft to be published. CEC was involved in conducting the research, review and revision of the manuscript, and approval of the final draft to be published. THC was involved in the design of the study, conducting the research, drafting of the manuscript and approval of the final draft to be published. NS was also involved in analysis and interpretation of the data.

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Patient consent Obtained.

Ethics approval The trial was approved by an independent Ethics Committee or Institutional Review Board, and all patients gave written informed consent for participation in the trial. This two-arm, randomised, multicentre, double-blind, placebo-controlled, parallel-group, phase III study was conducted in the USA, Canada and the UK (figure 1) at 34 sites (the MEASURE study).

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Supplementary Methods

Pulse Wave Velocity Assessments

The SphygmoCor system (AtCor, Inc., San Jose, CA)—including computer, software, relevant supplies and user’s manual—was provided to each site. Pulse wave velocity (PWV) measurements were obtained during baseline assessment and at weeks 2, 12 and 24. During the open-label follow-up, PWV measurements were obtained during weeks 36, 52 and 104. A detailed instruction manual was provided by AtCor to each site.

Patients were required to be supine quietly for 10 minutes before measurements were obtained. No interruptions or other study procedures were allowed during this 10-minute period. First, the patient’s supine blood pressure, measured by sphygmomanometry, was entered into the SphygmoCor system. A three-lead electrocardiogram was attached to the patient (each wrist, left leg), and the quality of the electrocardiogram was checked. Then carotid and femoral sites were palpated to determine the location of the strongest pulse, and distances from the suprasternal notch to the carotid pulse and to the femoral pulse were measured and entered into the SphygmoCor system. The tonometer was placed over the site, and its position was adjusted until a good-quality waveform was obtained. When a waveform signal of consistent pressure was obtained, the tonometer was held in position for at least 12 seconds; the data were then transferred to the system.

Quality indices indicated by the SphygmoCor system were assessed during the decision-making about whether to accept the measurement. The first PWV measurement that met quality control criteria was printed and retained as a source document. The distance from the center of the suprasternal notch directly to the carotid measurement site was measured; this was followed by similar measurement of the distance from the center of the suprasternal notch to the femoral measurement site. These values were entered into the system for calculation of the PWV values. The SphygmoCor database was then archived to a compact disk. Data were transferred to AtCor Medical (West Ryde, NSW, Australia) for quality control review. The initial data transfer took place immediately on the receipt and assembly of the SphygmoCor system. Ongoing data transfers occurred quarterly thereafter.
**Biomarker assays**

Secretory Phospholipase 2-IIA (sPLA2-IIA), oxidised LDL and D-dimer were determined using enzyme immunoassay (EIA) kits. For sPLA2-IIA (Cayman Chemical, Ann Arbor, MI, USA), a monoclonal capture antibody was coupled with detection by acetylcholinesterase (AChE)/Fab conjugate and DTNB detection. Oxidised LDL was measured by competitive assay (Mercodia, Uppsala, Sweden). The method makes use of a biotin-labeled monoclonal antibody, 4E6, first described by Holvoet, and detection by horseradish peroxidase (HRP)-conjugated streptavidin with 3,3′,5,5′-tetramethylbenzidine (TMB). D-dimer (American Diagnostica, Stamford, CT, USA) was assayed using D-dimer monoclonal capture and HRP-conjugated monoclonal with TMB detection. EDTA plasma fibrinogen (Kamiya Biomedical, Seattle, WA, USA), lipoprotein(a) (Denka Seikin, Niigata, Japan), hs-CRP (Roche Diagnostics, Indianapolis, IN, USA) and serum haptoglobin (Roche Diagnostics, Mannheim, Germany) were quantified using immunoturbidimetric assay kits. These assays were performed using a Roche Modular P autoanalyzer (Roche Diagnostics). For determination of HDL-associated SAA, serum HDL particles were isolated by polyethylene glycol 8000 (PEG-8000; Promega, Madison, WI) precipitation, as described by Chiba et al. Briefly, equal volumes of 13.0% PEG (P-4463; Sigma-Aldrich, St. Louis, MO) were mixed to precipitate non-HDL proteins and lipoproteins. After centrifugation for 5 minutes at 18,000g, supernate serum amyloid A was determined by EIA (Abzyme; Needham, MA, USA). An anti-SAA monoclonal capture and an HRP-conjugated polyclonal antibody were used with TMB detection. Paraoxonase activity was measured by modification of the method described by Haagen and Brock. The assay was performed using the Roche Modular P autoanalyzer, and enzyme activity was determined as the initial rate of p-nitrophenol formation from diethyl p-nitrophenyl phosphate at 415 nm.

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Supplementary Figure 1. Change in (A) ACR 20/50/70 response and (B) DAS28 response* at 12 and 24 weeks (TCZ vs placebo). ACR, American College of Rheumatology; DAS28, Disease Activity Score at 28 joints; MTX, methotrexate; TCZ, tocilizumab. *Data are presented as mean (SE).
**Supplementary Table 1.** Summary of adverse events with an incidence rate of at least 5% (week 24, safety population)

| Adverse event, n (%)                             | Placebo + MTX n = 62* | TCZ + MTX n = 70 |
|------------------------------------------------|-----------------------|------------------|
| Upper respiratory tract infection              | 6 (9.7)               | 8 (11.4)         |
| Diarrhoea                                      | 2 (3.2)               | 9 (12.9)         |
| Rheumatoid arthritis                           | 6 (9.7)               | 4 (5.7)          |
| Fatigue                                        | 7 (11.3)              | 2 (2.9)          |
| Urinary tract infection                        | 2 (3.2)               | 7 (10.0)         |
| Oropharyngeal pain                             | 1 (1.6)               | 6 (8.6)          |
| Rash                                           | 2 (3.2)               | 4 (5.7)          |
| Gastritis                                      | —                     | 5 (7.1)          |
| Nasopharyngitis                                | —                     | 5 (7.1)          |
| Sinusitis                                      | 1 (1.6)               | 4 (5.7)          |
| Vomiting                                       | 1 (1.6)               | 4 (5.7)          |
| Depression                                     | 4 (6.5)               | —                |
| Headache                                       | —                     | 4 (5.7)          |
| Mouth ulceration                               | —                     | 4 (5.7)          |

MTX, methotrexate; TCZ, tocilizumab.

Multiple occurrences of the same adverse event in one individual counted only once.

*One patient randomly assigned to placebo + MTX actually received one dose of tocilizumab and was therefore included in the TCZ group for the safety analyses.
Tocilizumab may increase the amount of cholesterol in the blood, but not the most damaging type of cholesterol

Some medicines used to treat inflammatory diseases such as rheumatoid arthritis may alter the balance of lipids and cholesterol in the blood.

INTRODUCTION
Rheumatoid arthritis is a chronic inflammatory disease that affects a person’s joints, causing pain and disability. People with rheumatoid arthritis have an increased risk of suffering from cardiovascular diseases such as heart attacks, heart failure or stroke. This is because the inflammation involved in rheumatoid arthritis can have an effect on other systems in the body, as well as the joints.

Recently, some biologic medicines that are used to treat rheumatoid arthritis have been associated with raised cholesterol levels – including tocilizumab, the drug investigated in this study. Tocilizumab is one of a group of drugs called biologics, and it works by blocking a molecule called interleukin-6 which is involved in inflammation.

Cholesterol can contribute to the development of cardiovascular problems, and so drugs that increase cholesterol levels may need to be avoided in patients who already have a naturally increased risk.

WHAT DID THE AUTHORS HOPE TO FIND?
The authors wanted to see whether there was a link between tocilizumab – a medicine that some patients take for rheumatoid arthritis – and the cholesterol and lipid levels in their blood. They hoped to help doctors to understand what changes occur in the blood of people who receive treatment with tocilizumab for their rheumatoid arthritis, since this may affect the likelihood of developing cardiovascular problems.

WHO WAS STUDIED?
The study included 132 patients with rheumatoid arthritis. All the patients were over the age of 18, had suffered from severe rheumatoid arthritis for at least 6 months, and had not seen an improvement with other drugs such as methotrexate. Patients who had already received a biologic drug were not able to enter into the study.

HOW WAS THE STUDY CONDUCTED?
This was a randomised, placebo-controlled trial, which means that patients were assigned by chance to one of two treatment groups to receive either tocilizumab (the active medicine) or a placebo (a dummy that has no active medicine in it). Using chance in this way means that the groups will be similar and will allow the variable or treatment under investigation to be compared objectively. During the treatment neither patients nor their doctors knew which group they were in. Both groups also received a drug called methotrexate.

For the next 6 months, regular blood tests were used to measure the lipid levels. The authors also used ultrasound to measure how well the blood vessels were working.

WHAT WERE THE MAIN FINDINGS OF THE STUDY?
The study found that tocilizumab does increase the amount of cholesterol in the blood. But importantly, these rises were not in the most damaging types of cholesterol, such as small low-density lipoprotein cholesterol or LDL-C, which may be more associated with the development of cardiovascular problems than larger high-density lipoprotein cholesterol, or HDL-C. They also found that there was an increase seen in a group of proteins that are normally associated with the protection of blood vessels and reduced vascular risk, and there was a 30% decrease in lipoprotein(a) which has previously been found to be associated with vascular events. It will be important to work out what these findings mean for patients in the long term.

There were no clear results from the ultrasound part of the study.

The authors also found that tocilizumab improved the signs and symptoms of rheumatoid arthritis over 24 weeks in the study and was better than placebo; these results were consistent with what has previously been reported.

ARE THESE FINDINGS NEW?
Yes – this is the first time anyone has looked at the blood of patients receiving tocilizumab to work out how it affects these markers.
HOW RELIABLE ARE THE FINDINGS?
There are some limitations which may affect how reliable the findings are. The study was conducted in a small number of patients over a short time, which may mean that it is not reflective of the long periods that rheumatoid arthritis patients take their medicines for. There were some technical problems with the ultrasound measurements which meant that not all of the results could be used. Also, the authors measured only lipids in the blood and did not link these to whether patients went on to develop cardiovascular problems – this may be an area that needs further study.

Although the findings are reliable in terms of understanding how tocilizumab changes the levels of certain markers in the blood, it is not possible to draw predictions on the safety of tocilizumab from this.

WHAT DO THE AUTHORS PLAN ON DOING WITH THIS INFORMATION?
These results will be shared with other doctors and academics to inform them of the findings. There is a follow-up study planned which will look more closely at what causes the changes to happen.

WHAT DOES THIS MEAN FOR ME?
Patients with rheumatoid arthritis who are taking tocilizumab should have their cholesterol levels and blood pressure checked as agreed in their care plan, and should report any symptoms that they are concerned about to their doctor. It is important that patients do not stop taking their medicine without talking to their doctor first. More studies will be needed to confirm these findings and to make clear links between the effects and cardiovascular risks and for recommendations to be made to doctors.

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