SCIL-STROKE (Subcutaneous Interleukin-1 Receptor Antagonist in Ischemic Stroke)

DOI:
10.1161/STROKEAHA.118.020750

Document Version
Accepted author manuscript

Title: SUBCUTANEOUS INTERLEUKIN-1 RECEPTOR ANTAGONIST IN ISCHEMIC STROKE (SCIL-STROKE): A RANDOMIZED CONTROLLED PHASE 2 TRIAL

Manuscript number: STROKE/2018/020750DR1

Author(s): Craig Smith

Sharon Hulme

Andy Vail

Calvin Heal

Adrian Parry-Jones

Sylvia Scarth

Karen Hopkins

Margaret Hoadley

Stuart Allan

Nancy Rothwell

Stephen Hopkins

Pippa Tyrrell

SUBCUTANEOUS INTERLEUKIN-1 RECEPTOR ANTAGONIST IN ISCHEMIC STROKE (SCIL-STROKE): A RANDOMIZED CONTROLLED PHASE 2 TRIAL

Craig J Smith MD[1,2], Sharon Hulme RGN BSc[2], Andy Vail MSc[3], Calvin Heal MSc[3], Adrian R Parry-Jones PhD[1,2], Sylvia Scarth BSc[2], Karen Hopkins† BSc[2], Margaret Hoadley PhD[2], Stuart M Allan PhD[4], Nancy J Rothwell DSc[4], Stephen J Hopkins PhD[2], Pippa J Tyrrell MD[1,2]

[1]Greater Manchester Comprehensive Stroke Centre, Manchester Academic Health Science Centre, Salford Royal NHS Foundation Trust, UK; [2]Division of Cardiovascular Sciences, University of Manchester, Manchester, UK; [3]Centre for Biostatistics, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK; [4]Division of Neuroscience and Experimental Psychology, University of Manchester, Manchester, UK

†Died on 2nd May 2017

Corresponding author: Professor Craig J Smith

+44(0)161 206 0623, craig.smith-2@manchester.ac.uk

Cover title: IL-1 receptor antagonist in ischemic stroke

Tables and Figures: Tables 1; Figures 4

Online-only figures and tables: Online Tables 5; Online Figures 2

Keywords: Acute Ischemic Stroke; Clinical Trial; Interleukin-1 Receptor Antagonist; Interleukin-6

Subject terms: Ischemic Stroke; Inflammation; Treatment

Word count: 4994

Background and Purpose

The pro-inflammatory cytokine interleukin-1 (IL-1) has a deleterious role in cerebral ischemia, which is attenuated by IL-1 receptor antagonist (IL-1Ra). IL-1 induces peripheral inflammatory mediators, such as interleukin-6 (IL-6), which are associated with worse prognosis after ischemic stroke. We investigated whether subcutaneous (SC) IL-1Ra reduces the peripheral inflammatory response in acute ischemic stroke.

Methods

SCIL-STROKE was a single-center, double-blind, randomized, placebo-controlled phase 2 trial of SC IL-1Ra (100mg administered twice daily for three days) in patients presenting within 5h of ischemic stroke onset. Randomization was stratified for baseline NIHSS score and thrombolysis. Measurement of plasma IL-6 and other peripheral inflammatory markers was undertaken at five time points. The primary outcome was difference in concentration of log(IL-6) as area under the curve to Day 3. Secondary outcomes included exploratory effect of IL-1Ra on three month outcome with the modified Rankin Scale (mRS).

Results

We recruited 80 patients (mean age 72, median NIHSS 12) of whom 73% received intravenous thrombolysis with alteplase. IL-1Ra significantly reduced plasma IL-6 ($p<0.001$) and plasma C-reactive protein ($p<0.001$). IL-1Ra was well-tolerated with no safety concerns. Allocation to IL-1Ra was not associated with a favorable outcome on mRS: OR (95% CI) = 0.67 (0.29 to 1.52); p=0.34. Exploratory mediation analysis suggested that IL-1Ra improved clinical outcome by reducing inflammation, but there was a statistically significant, alternative mechanism countering this benefit.

Conclusions

IL-1Ra reduced plasma inflammatory markers which are known to be associated with worse clinical outcome in ischemic stroke. SC IL-1Ra is safe and well-tolerated. Further

experimental studies are required to investigate efficacy and possible interactions of IL-1Ra with thrombolysis.

Clinical Trial Registration

URL: http//www.isrctn.com. Unique identifier: ISRCTN74236229

Introduction

Inflammation is a major contributor to the pathophysiology and outcome of cerebral ischemia. The pro-inflammatory cytokine interleukin-1 (IL-1) is up-regulated rapidly in experimental stroke, and worsens ischemic injury in pre-clinical models of cerebral ischemia.[1] IL-1 induces peripheral inflammatory markers, such as interleukin-6 (IL-6), which are associated with worse prognosis after ischemic stroke.[2] The endogenous IL-1 receptor antagonist (IL-1Ra) blocks all known actions of IL-1 by competitively inhibiting the IL-1 receptor 1, without any reported agonist activity.[3] IL-1Ra markedly attenuates experimental brain injury in experimental cerebral ischemia, showing efficacy in aged and comorbid animals, when administered peripherally by subcutaneous (SC) or intravenous (IV) routes, and with delayed administration.[1] The efficacy of IL-1Ra in experimental middle cerebral artery occlusion (MCAO) has been reproduced in a cross-laboratory study using a range of outcome measures, including longer-term functional recovery.[4] A recently updated meta-analysis of pre-clinical stroke studies showed that IL-1Ra treatment reduced infarct volume by 36% in pooled data from 1283 animals.[5]

We have reported previously that IV IL-1Ra administered over 72 h in a placebo-controlled phase 2 study of patients with acute stroke was safe and significantly reduced plasma inflammatory markers.[6] IV IL-1Ra penetrated cerebrospinal fluid (CSF) at experimentally therapeutic concentrations in patients with acute aneurysmal subarachnoid hemorrhage (aSAH),[7,8] and reduced CSF and plasma IL-6 concentrations.[9] IL-1Ra is now available as a commercial SC formulation used to treat rheumatoid arthritis, which is cheaper and more practical to administer. After pharmacokinetic modelling of SC treatment, based on therapeutic concentrations of IV IL-Ra, we recently undertook a phase 2 trial of SC IL-1Ra in

acute aSAH in which SC IL-1Ra was safe and significantly reduced plasma IL-6 and C-reactive protein (CRP) concentrations.[10]

Our previous trial of IV IL-1Ra in ischemic stroke was undertaken prior to the UK licence and therefore widespread clinical use of alteplase in the UK. In the present study we investigated whether SC IL-1Ra reduces the peripheral inflammatory response in acute ischemic stroke, alongside standard of care thrombolysis when clinically indicated. Our primary objective was to determine the effect of SC IL-1Ra on plasma IL-6 to Day 3. Secondary objectives included obtaining exploratory clinical outcome data to inform subsequent phase 3 trial design.

Methods

In line with AHA Journals' implementation of the Transparency and Openness Promotion (TOP), the data that support the findings of this study are available from the corresponding author upon reasonable request.

Design and setting

SCIL-STROKE was a single-center, double-blind, randomized, placebo-controlled phase 2 clinical trial undertaken at the Greater Manchester Comprehensive Stroke Centre (CSC) at Salford Royal NHS Foundation Trust (SRFT). Approvals were obtained from Greater Manchester South NHS Research Ethics Committee, the UK Medicines and Healthcare Products Regulatory Agency (MHRA) and SRFT Research and Development.

Participants

Full eligibility criteria are presented in Table I in the online-only Data Supplement. Patients presenting with confirmed ischemic stroke, aged ≥ 18 y, with a National Institutes of Health Stroke Scale (NIHSS) score between 4 and 26 and within 5 h of symptom onset were eligible. Baseline NIHSS, drug history, routine blood tests and CT head were undertaken as part of urgent clinical assessment and informed eligibility screening. Hyperacute research practitioners recruited participants between 0700 and 2100 seven days a week, although a screening log was kept of all potentially eligible patients. If capacity to consent to participation was absent, consent was sought from a personal consultee if available or a professional consultee (a senior clinician independent of the study). Baseline assessment was then completed, including past medical history, modified Rankin Scale (mRS) in the month preceding ictus, vascular risk factors and vital signs.

Intervention

Participants received six doses of either 100mg SC recombinant human IL-1Ra (anakinra) or placebo. The first dose was started within 6 h of symptom onset. Five further doses were

administered starting at the next administration time point (07:00 or 19:00 ± 2 h), at least 6 h after the first dose and continued every 12 h until completion of the six doses. This regimen was derived from pharmacokinetic model and tolerability data from Amgen, Inc., subsequently validated in our SC IL-1Ra trial in aSAH.[10]

Randomization and blinding

Randomization (1:1) was undertaken using an independent third-party, web-based service, stratified for stroke severity (NIHSS <13 or ≥13) and IV thrombolysis.

Venous blood for plasma inflammatory markers

Venous blood was drawn prior to consent in potentially eligible patients, supported by our regulatory approvals, to avoid delays in obtaining the baseline pre-randomisation sample (Day 0). This sample was discarded if patients were not included. Blood was also drawn on Days 1, 2, 3 and between Days 5 to 7. The Day 5 to 7 sample was discontinued following blinded review by the Trial Steering Committee after recruitment of the first 58 participants, as it had only been obtained in 51% of participants (largely due to participant repatriation to their base hospital after Day 3).

Processing and analyses of blood samples

Venous blood was processed and analysed at SRFT. Blood was collected in EDTA, centrifuged at 2000 g at 4° C for 15 min, and the plasma frozen at -70° C**.** Plasma IL-6 and IL-1Ra were measured using enzyme linked immunosorbent assays (ELISA) and CRP and von-Willebrand Factor (vWF) were measured in a duplex assay using Luminex bead technology (see online-only Data Supplement). Laboratory staff were blinded to treatment allocation, except for the individual measuring plasma IL-1Ra. The concentrations of IL-6, CRP and vWF were transformed using the natural logarithm prior to analysis.

Outcomes

The primary outcome measure was the area under the curve (AUC) for the log-transformed concentration of plasma IL-6 between Day 1 and Day 3. Only participants with a pre-randomisation sample (Day 0) and at least two of the three subsequent blood samples (Day 1, 2 or 3) were eligible for the primary analysis. Laboratory secondary outcomes were the AUC for plasma log(CRP) and log(vWF). Clinical blood tests and other investigations were undertaken at the discretion of the clinical team and reviewed by the research team. Serious adverse events (SAEs) and adverse events (AEs) were recorded daily to Day 3, and also at Day 30 (± 7d). All brain imaging was reported by neuroradiologists independent of the study, who were blinded to test treatment allocation. Hemorrhagic transformation was classified using the European Cooperative Acute Stroke Study (ECASS) criteria,[11] i.e. hemorrhagic infarction (HI) 1 or 2 and parenchymal hemorrhage (PH) 1 or 2. The clinical secondary outcome was the mRS at three months, undertaken by a single, trained rater blinded to treatment allocation, either face-to-face or by telephone.

Sample size

Our previous Phase 2 trial of IV IL-1Ra in patients with acute ischemic stroke showed a difference (SD) in log(CRP) of 0.8 (1.0) at 3 days.[6] Based on these data, 30 patients were required per group for 80% power at the 5% significance level to detect a difference of 0.75 SD in the primary outcome. We therefore aimed to recruit up to 80 patients in total to allow for loss to follow-up. We also considered that complete 3-month mRS data on 50 participants would be adequate for estimation of parameters to inform a phase 3 sample size calculation.

Statistical methods

All analyses were pre-specified in a statistical analysis plan before study completion and unblinding of the dataset. Demographic and clinical data at baseline were tabulated by allocated test treatment. The primary analysis used linear regression to control for the randomization stratification criteria and for the baseline value of log(IL-6). Where one of the

Day 1 to Day 3 blood samples was unavailable, imputation was undertaken. The fitted value from linear regression of log(IL-6) on 'Day' was substituted for the missing value. The assumption of linearity over this time period was assessed graphically but was anticipated to be reasonable based on our previous aSAH study data. These linear regression analyses were repeated for plasma CRP and vWF, but adjusting each for its own baseline value. Ordinal logistic regression was used to analyse mRS, adjusting for the stratification variables. We also undertook mediation analysis to explore the hypothesized causal pathway from treatment [IL-1Ra] through mediator [AUC log(IL-6)] to outcome [mRS].[12] As this method is not yet developed for ordinal outcomes, we first dichotomized the mRS as 0 to 2 versus 3 to 6. All analyses were undertaken in Stata version 14.

Results

Of 742 patients screened between 22nd March 2014 and 31st October 2016, 207 met the eligibility criteria. Of these, 118 were approached and 80 (68%) were recruited (Fig. 1). One patient deteriorated rapidly after randomization (to IL-1Ra) and was withdrawn prior to receiving any test treatment. Baseline characteristics of the recruited patients are shown in Table 1. Whilst the majority of participants (73%) received IV alteplase, the onset to needle (OTN) time was shorter in the placebo group. The interval from stroke onset to initiation of test treatment was not different between the two allocation groups.

Sixty three participants (n=35 placebo; n=28 IL-1Ra) had sufficient blood samples for the primary analysis (Fig. 1), thereby meeting our planned sample size. IL-1Ra prevented the rise in plasma IL-6 concentrations observed in the placebo group (Fig. 2(a)) and significantly reduced the AUC plasma log(IL-6) (Fig. 3(a)) in the adjusted linear regression analyses ($p<0.001$) (Table II in the online-only Data Supplement). *Post-hoc* sensitivity analyses taking into account imputation, and its potential interaction with the treatment effect, made no substantive difference to these findings (Table III in the online-only Data Supplement). A similar effect of IL-1Ra treatment was observed for plasma CRP concentrations (Fig. 2(b), Fig. 3(b) and Table IV in the online-only Data Supplement), but there was no observed difference in plasma vWF concentrations (Fig. 2(c) and Fig. 3(c)).

There were 16 SAEs in 14 patients (11 SAEs in nine allocated placebo and five SAEs in five allocated IL-1Ra) and 22 AEs in 19 patients (14 AEs in 12 allocated placebo and eight AEs in seven allocated IL-1Ra) (Table V in the online-only Data Supplement). Hemorrhagic transformation occurred in six patients allocated IL-1Ra (HI1 in one; PH1 in two; PH2 in three) and four allocated placebo (HI1 in two; PH1 in two). There were nine systemic

infections in the placebo group compared to three in the IL-1Ra group (for pneumonia or chest infection, seven v one). There were no reported injection-site reactions.

Final three month mRS was available in 75 participants (94%). Mortality was similar between the allocation groups (Fig. 1). More participants achieved a three month mRS of 0-2 in the placebo group (51%) compared to the IL-1Ra group (42%) (Figure I (a) in the online-only Data Supplement). In the adjusted ordinal logistic regression analyses, allocation to IL-1Ra was not associated with a favorable outcome on the mRS: OR (95% CI) = 0.67 (0.29 to 1.52), p=0.34). Further adjustment for pre-stroke mRS and thrombolysis OTN time in the regression model did not materially alter these findings (data not shown).

The mediation analysis (Fig. 4) suggested that, whilst taking baseline plasma IL-6 concentrations into account, IL-1Ra reduced the odds of a poor mRS outcome via the expected mechanism of reducing plasma IL-6 (OR (95% CI) =0.42 (0.19 to 0.94), p=0.04). By contrast, there was a negative 'residual' effect, in that IL-1Ra increased the odds of a poor mRS score (OR (95%CI) = 4.58 (1.19 to 17.71), p=0.03) by some other pathway or mechanism. Given that the majority of patients received thrombolysis, we undertook further *post-hoc* analyses adjusting the mediation approach for additional covariates, including baseline severity and thrombolysis in various categorizations (e.g. early versus late). None of these exploratory analyses substantively affected the findings.

Discussion

IL-1 mediates inflammatory and immune responses in both the CNS and periphery which are strongly implicated in cerebral ischemic injury and clinical outcome.[1] As downstream biomarkers of IL-1 induction, elevated plasma IL-6 and CRP concentrations are consistently predictive of poor clinical outcome in the acute phase of ischemic stroke.[2,13,14] Here, we have shown that treatment with SC IL-1Ra in hyperacute ischemic stroke significantly reduced plasma concentrations of IL-6 and CRP over the first three days. These findings are consistent with reduced concentrations of these plasma inflammatory markers observed in our previous studies of SC IL-1Ra in aSAH[10] and IV IL-1Ra in acute stroke and aSAH.[6,9] To our knowledge, such robust and consistent reduction of downstream peripheral inflammatory markers has not been demonstrated with other candidate anti-inflammatory drugs for acute stroke.[15]

The mechanisms by which peripherally-administered IL-1Ra reduces plasma IL-6 concentrations are yet to be fully characterised. IL-1 and IL-6 are upregulated rapidly in the ischemic hemisphere after MCAO[16-18] and their concentrations are elevated acutely in the CSF of ischemic stroke patients.[19] Elevated plasma IL-6 concentrations precede those in the ischemic hemisphere in experimental MCAO,[20] yet production of IL-1 and IL-6 by peripheral blood leucocytes within hours of stroke onset is negligible.[21] Hence, as yet undetermined, peripheral cellular sources may produce IL-6 in response to cerebral ischemia. Peripherally administered IL-1Ra crosses the blood-CSF barrier and blood-brain-barrier (BBB) after SAH[22] and it is therefore likely that SC IL-1Ra reduces plasma IL-6 by inhibiting it's induction by IL-1 both in the ischemic brain, and in the peripheral circulation.

We were surprised to observe a 95% confidence interval for clinical outcome that excluded a major benefit of treatment with IL-1Ra. This contrasts with our previous studies both of SC IL-1Ra in aSAH and IV IL-1Ra in stroke. Indeed, IL-1Ra in aSAH has now progressed to a multi-center phase 3 trial (NCT03249207). A *post-hoc* individual participant data meta-analysis combining our present data in ischemic stroke patients with our previous Phase 2 trial investigating IV IL-1Ra in a similar population (Figure II in the online-only Data Supplement) did not alter this conclusion. Whilst differences in the distribution of mRS scores between IL-1Ra and placebo were observed when comparing the previous IV trial and present SC trial (Figure I in the online-only Data Supplement), these should be interpreted cautiously as secondary analyses in relatively small sample sizes.

We used mediation analysis, which to our knowledge is a novel approach in acute stroke trials. The mediation analysis suggested that IL-1Ra improved the odds of a favorable clinical outcome by reducing plasma IL-6 concentrations (the mediator variable), indicative of upstream IL-1 blockade, as expected. However, the mediation analysis revealed an additional, "residual" negative pathway with IL-1Ra treatment, contributing to overall clinical outcome. Whilst these exploratory secondary analyses need to be interpreted with caution, they are useful in hypothesis generation when considering future studies of IL-1Ra in ischemic stroke. Similar mediation analyses in our recent aSAH trial also confirmed a favorable effect of IL-1Ra on clinical outcome through lowering plasma IL-6 concentrations, but with no residual negative effect. Similarly, re-analysis of our IV IL-1Ra in stroke study (data not published) did not suggest any residual effect. One clear difference is that this is the first of our IL-1Ra studies in which thrombolysis has been administered. The importance of potential interactions between candidate treatments and thrombolysis in acute stroke trials has previously been highlighted.[23] Tissue plasminogen activator (tPA) has a range of deleterious

effects in cerebral ischemia, several of which are independent of it's fibrinolytic activity.[24] There are few data concerning combined treatment with IL-1Ra plus tPA in pre-clinical studies of ischemic stroke,[4] but there is no suggestion of any adverse interaction. IL-1Ra might be expected to augment the beneficial effects of tPA treatment by reducing the deleterious effects of reperfusion injury, tPA-induced neurotoxicity and neuro-inflammation.[25,26] Conversely, IL-1 increases levels of endogenous plasminogen activator inhibitor-1,[27] the principal physiological inhibitor of tPA. IL-1 blockade combined with tPA in ischemic stroke could therefore potentiate excessive fibrinolysis or other deleterious effects. We observed no differences in hemorrhagic transformation but as this is a relatively small sample size, a difference cannot be excluded from our present data. Finally, the transcription factor high mobility group box-1 (HMGB-1), which is up-regulated rapidly after cerebral ischemia,[28] augments tPA-induced fibrinolysis and protects against tPA-induced neurotoxicity and BBB damage in experimental stroke.[29] As IL-1 up-regulates HMGB-1 in mononuclear cells,[30] IL-1 blockade might impact negatively on these apparent protective effects of HMGB-1 in the setting of thrombolysis with tPA.

Our study has several strengths. It was designed in line with consensus recommendations for phase 2 clinical trials of candidate drugs for acute ischemic stroke.[31,32] Our dosing regimen was based on robust pharmacokinetic modelling and known pre-clinical therapeutic concentrations. A relevant surrogate outcome measure was selected for the primary outcome, measured using well-established methods and confirming proof of concept. Our design allowed us to test IL-1Ra alongside thrombolysis with IV alteplase to explore combination therapy.[23,32] We achieved our planned sample size for the primary analyses and employed ordinal analysis of the mRS secondary outcome. Mediation analysis enabled hypothesis generation to inform future experimental and clinical studies.

We also acknowledge several limitations of our trial. Recruitment was limited to a single-center which may have implications for generalizability. Inclusion of patients receiving IV thrombolysis as part of urgent clinical care is likely to have introduced inevitable delays in administration of test treatment. However, although onset to test treatment was on average 4.4 h, we still observed significant reductions in plasma IL-6. Our study design did not include evaluation of pre- and post- treatment angiography to determine site of arterial occlusion and whether spontaneous or fibrinolytic reperfusion occurred in the context of allocation to IL-1Ra or placebo. Likewise, we did not undertake pre- and post- treatment MR imaging to evaluate infarct expansion, oedema or infarct volume. These may have provided useful insights in secondary analyses although interpretation is likely to have been limited given our relatively small sample size. Our regional clinical service model meant that some participating patients were repatriated to their local hospital prior to completion of blood sampling, which limited availability of the day 5 to 7 sample. Finally, the mediation analyses were necessarily restricted to those patients with sufficient plasma IL-6, which limited sample size further.

Summary

We have demonstrated that SC IL-1Ra significantly reduced plasma inflammatory markers that are known to be associated with a worse outcome after ischemic stroke. In hypothesis-generating mediation analyses, SC IL-1Ra positively influenced outcome by the expected mechanism of reducing plasma concentrations of IL-6, but there was a residual negative effect which may represent an interaction with alteplase. Further experimental stroke studies are required to fully investigate efficacy and mechanisms of IL-1Ra in the presence of thrombolytic agents.

Acknowledgements

We are grateful to SOBI, for supplying SC IL-1Ra and placebo, and our clinical colleagues and LCRN acute research delivery team practitioners at the Greater Manchester CSC. We thank Dr Amit Herwadkar (consultant neuroradiologist) for applying the ECASS criteria to the 24h CT scans. We are also grateful to the independent members of the Trial Steering Committee, Professor Tom Robinson (University of Leicester) and Professor David Crossman (University of St Andrews); and the Data Monitoring Committee, Dr Steven Lane (University of Liverpool), Dr Allison Morton (University of Sheffield) and Dr John Bamford, DMC Chair (University of Leeds).

Sources of Funding

This study was funded by The Stroke Association (TSA 2012/08).

Conflicts of Interest/Disclosures

The funder had no role in the design or conduct of the study, the data analyses or interpretation, or the decision to publish the findings. SOBI supplied the test treatment and reviewed the content of the manuscript prior to submission for publication.

References

1. Sobowale OA, Parry-Jones AR, Smith CJ, Tyrrell PJ, Rothwell NJ, Tyrrell PJ. Interleukin-1 in stroke: from bench to bedside. *Stroke.* 2016;47:2160-2167.
2. Bustamante A, Sobrino T, Giralt D, García-Berrocoso T, Llombart V, Ugarriza I, et al. Prognostic value of blood interleukin-6 in the prediction of functional outcome after stroke: a systematic review and meta-analysis. *J Neuroimmunol*. 2014;274:215-224.
3. Arend WP, Welgus HG, Thompson RC, Eisenberg SP. Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. *J Clin Invest.* 1990;85:1694-1697.
4. Mayasami S, Wong R, Pradillo JM, Denes A, Dhungana H, Malm T, et al. A cross-laboratory preclinical study on the effectiveness of interleukin-1 receptor antagonist in stroke. *J Cereb Blood Flow Metab*. 2016;36:596-605.
5. McCann SK, Cramond F, Macleod MR, Sena ES. Systematic review and meta-analysis of the efficacy of interleukin-1 receptor antagonist in animal models of stroke: an update. *Transl Stroke Res*. 2016;7:395-406.
6. Emsley HC, Smith CJ, Georgiou RF, Vail A, Hopkins SJ, Rothwell NJ, et al. A randomised phase II study of interleukin-1 receptor antagonist in acute stroke patients. *J Neurol Neurosurg Psychiatry*. 2005;76:1366-1372.
7. Clark SR, McMahon CJ, Gueorguieva I, Rowland M, Scarth S, Georgiou R, et al. Interleukin-1 receptor antagonist penetrates human brain at experimentally therapeutic concentrations. *J Cereb Blood Flow Metab*. 2008;28:387-394.
8. Galea J, Ogungbenro K, Hulme S, Greenhalgh A, Aarons L, Scarth S, et al. Intravenous anakinra can achieve experimentally effective concentrations in the

central nervous system within a therapeutic time window: results of a dose-ranging study. *J Cereb Blood Flow Metab*. 2011;31:439-447.

9. Singh N, Hopkins SJ, Hulme S, Galea J, Hoadley M, Vail A, et al. The effects of intravenous interleukin-1 receptor antagonist on inflammatory markers in cerebrospinal fluid after subarachnoid haemorrhage: a phase II randomised controlled trial. *J Neuroinflamm*. 2014;11:1.
10. Galea J, Ogungbenro K, Hulme S, Patel H, Scarth S, Hoadley M, et al. Reduction of inflammation after administration of interleukin-1 receptor antagonist following aneurysmal subarachnoid haemorrhage: results of the Subcutaneous Interleukin-1Ra in SAH (SCIL-SAH) study. *J Neurosurg*. 2017;24:1-9.
11. Fiorelli M, Bastianello S, von Kummer R, del Zoppo GJ, Larrue V, Lesaffre E, et al. Hemorrhagic transformation within 36 hours of a cerebral infarct: relationships with early clinical deterioration and 3-month outcome in the European Cooperative Acute Stroke Study I (ECASS I) cohort. *Stroke*. 1999;30:2280-2284.
12. Petersen ML, Sinisi SE, Van Der Laan MJ. Estimation of direct causal effects. *Epidemiology*. 2006;17:276-284.
13. Whiteley W, Jackson C, Lewis S, Lowe G, Rumley A, Sandercock P, et al. Inflammatory markers and poor outcome after stroke: a prospective cohort study and systematic review of interleukin-6. *PLoS Med*. 2009;6:e1000145.
14. Hou D, Liu J, Feng R, Gao Y, Wang Y, Wu J. The role of high-sensitivity C-reactive protein levels in functional outcomes in patients with large-artery atherosclerosis and small-artery occlusion. *Neurol Res*. 2017;27:1-7.
15. Smith CJ, Denes A, Tyrrell PJ, Di Napoli M. Phase II anti-inflammatory and immune-modulating drugs for acute ischaemic stroke. *Expert Opin Investig Drugs*. 2015;24:623-643.

16. Davies CA, Loddick SA, Toulmond S, Stroemer RP, Hunt J, Rothwell NJ. The progression and topographic distribution of interleukin-1 beta expression after permanent middle cerebral artery occlusion in the rat. *J Cereb Blood Flow Metab*. 1999;19:87-98.

17. Suzuki S, Tanaka K, Nogawa S, Nagata E, Ito D, Dembo T, et al. Temporal profile and cellular localization of interleukin-6 protein after focal cerebral ischemia in rats. *J Cereb Blood Flow Metab*. 1999;19:1256-1262.

18. Luheshi NM, Kovács KJ, Lopez-Castejon G, Brough D, Denes A. Interleukin-1α expression precedes IL-1β after ischemic brain injury and is localised to areas of focal neuronal loss and penumbral tissues. *J Neuroinflammation*. 2011;8:186.

19. Tarkowski E, Rosengren L, Blomstrand C, Wikkelsö C, Jensen C, Ekholm S, et al. Early intrathecal production of interleukin-6 predicts the size of brain lesion in stroke. *Stroke*. 1995;26:1393-1398.

20. Chapman KZ, Dale VQ, Denes A, Bennett G, Rothwell NJ, Allan SM, et al. A rapid and transient peripheral inflammatory response precedes brain inflammation after experimental stroke. *J Cereb Blood Flow Metab*. 2009;29:1764-1768.

21. Emsley H, Smith CJ, Gavin CM, Georgiou RF, Vail A, Barberan E, et al. Clinical outcome following acute ischaemic stroke relates to both activation and autoregulatory inhibition of cytokine production. *BMC Neurol*. 2007;7:5.

22. Gueorguieva I, Clark SR, McMahon CJ, Scarth S, Rothwell NJ, Tyrrell PJ, et al. Pharmacokinetic modelling of interleukin-1 receptor antagonist in plasma and cerebrospinal fluid of patients following subarachnoid haemorrhage. *Br J Clin Pharmacol*. 2008;65:317-25.

23. Albers GW, Goldstein LB, Hess DC, Wechsler LR, Furie KL, Gorelick PB, et al. Stroke Treatment Academic Industry Roundtable (STAIR) recommendations for

maximizing the use of intravenous thrombolytics and expanding treatment options with intra-arterial and neuroprotective therapies. *Stroke*. 2011;42:2645-2650.

24. Vivien D, Gauberti M, Montagne A, Defer G, Touzé E. Impact of tissue plasminogen activator on the neurovascular unit: from clinical data to experimental evidence. *J Cereb Blood Flow Metab*. 2011;31:2119-2134.
25. Benchenane K, Castel H, Boulouard M, Bluthé R, Fernandez-Monreal M, Roussel BD, et al. Anti-NR1 N-terminal-domain vaccination unmasks the crucial action of tPA on NMDA-receptor-mediated toxicity and spatial memory. *J Cell Sci*. 2007;120:578-85.
26. Zhang C, An J, Haile WB, Echeverry R, Strickland DK, Yepes M. Microglial low-density lipoprotein receptor-related protein 1 mediates the effect of tissue-type plasminogen activator on matrix metalloproteinase-9 activity in the ischemic brain. *J Cereb Blood Flow Metab*. 2009;29:1946-1954.
27. Jansen PM, Boermeester MA, Fischer E, de Jong IW, van der Poll T, Moldawer LL, et al. Contribution of interleukin-1 to activation of coagulation and fibrinolysis, neutrophil degranulation, and the release of secretory-type phospholipase A2 in sepsis: studies in nonhuman primates after interleukin-1 alpha administration and during lethal bacteremia. *Blood*. 1995;86:1027-34.
28. Liesz A, Dalpake A, Mrackso E, Antoine DJ, Roth S, Zhou W, et al. DAMP signalling is a key pathway inducing immune modulation after brain injury. *J Neurosci*. 2015;35:583-598.
29. Roussel BD, Mysiorek C, Rouhiainen A, Jullienne A, Parcq J, Hommet Y, et al. HMGB-1 promotes fibrinolysis and reduces neurotoxicity mediated by tissue plasminogen activator. *J Cell Sci*. 2011;124:2070-2076.

30. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science*. 1999;285:248-251.

31. Stroke Therapy Academic Industry Roundtable II (STAIR-II). Recommendations for clinical trial evaluation of acute stroke therapies. *Stroke*. 2001;32:1598-606.

32. Fisher M, Stroke Therapy Academic Industry Roundtable. Recommendations for advancing development of acute stroke therapies: Stroke Therapy Academic Industry Roundtable 3. *Stroke*. 2003;34:1539-1546.

Figure legends

Figure 1: CONSORT diagram illustrating study screening, recruitment and follow-up

Figure 2: Serial log concentrations of plasma (a) interleukin-6 (IL-6); (b) C-reactive protein (CRP); and (c) von-Willebrand Factor (vWF) from pre-randomisation (Day 0) for patients receiving interleukin-1 receptor antagonist (IL-1Ra) or placebo. Boxes show median (horizontal line) between the first and third quartiles, whiskers represent the minimum and maximum values and outliers are shown as closed circles or diamonds

Figure 3: Distribution of Area Under the Curve (AUC) plasma Log-transformed IL-6 (a), CRP (b) and vWF (c) by treatment allocation

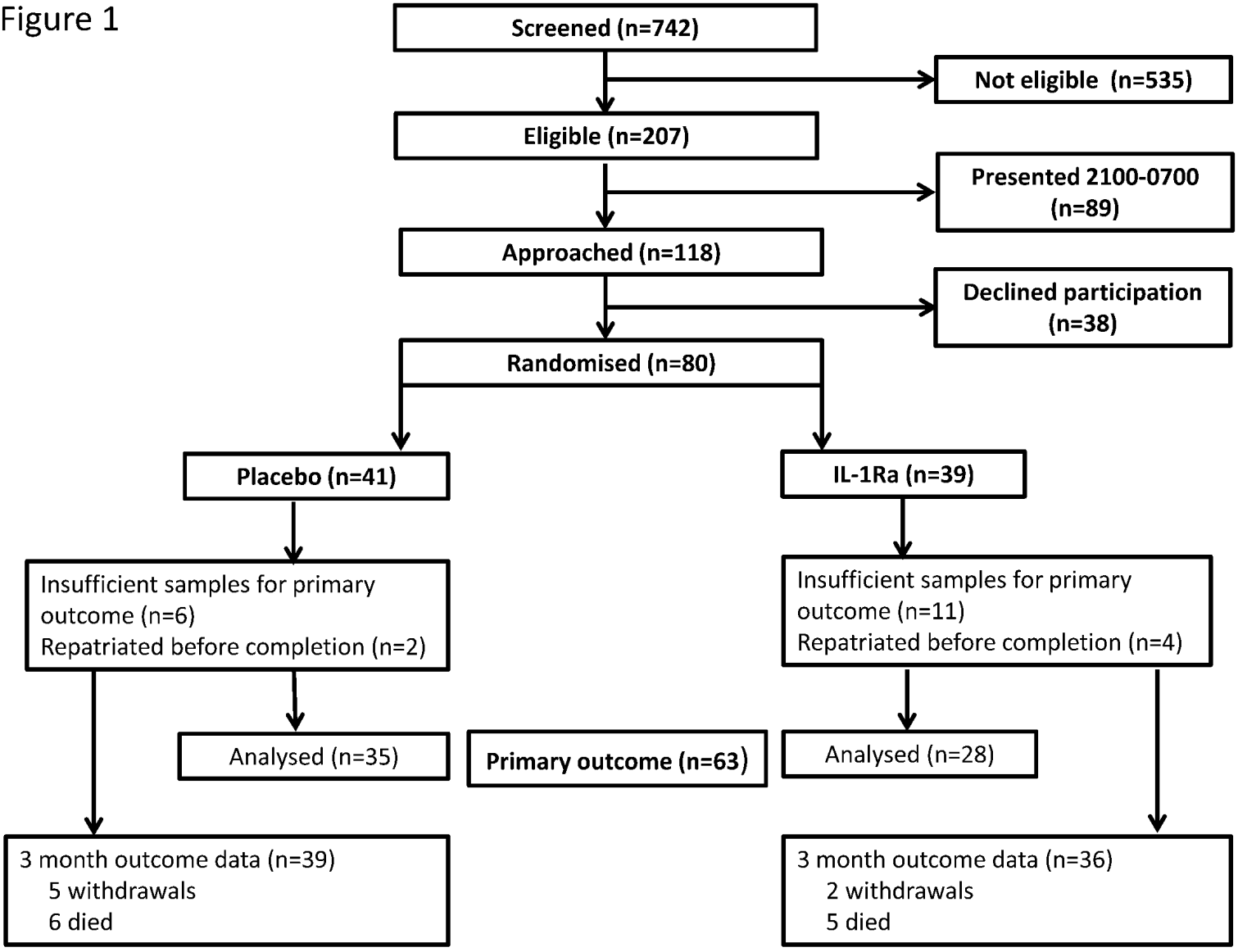
Figure 4: Summary of mediation analysis representing the causal pathway between the test treatment [IL-1Ra], mediator variable [AUC log(IL-6)] and outcome [mRS]

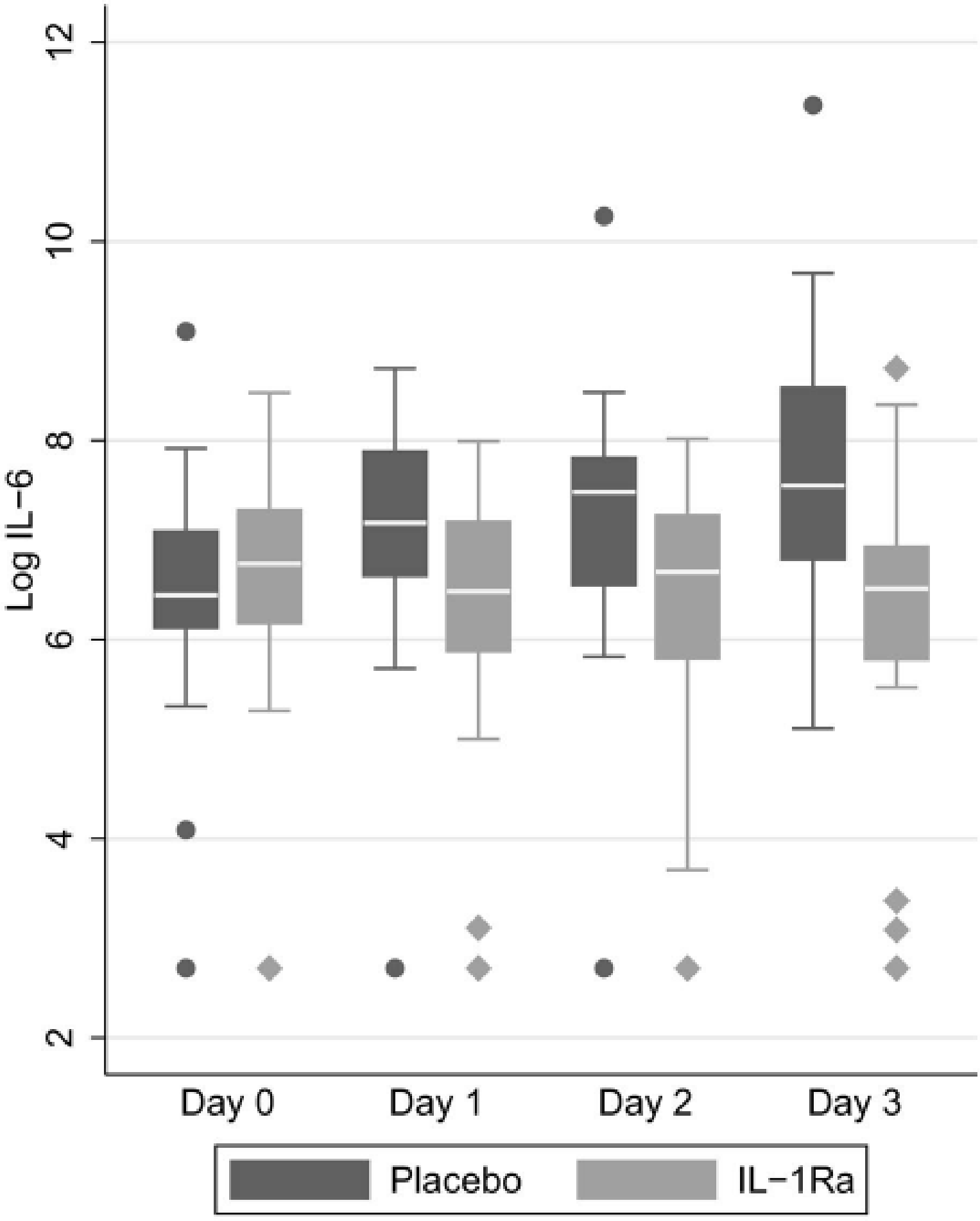
Table 1: Baseline characteristics

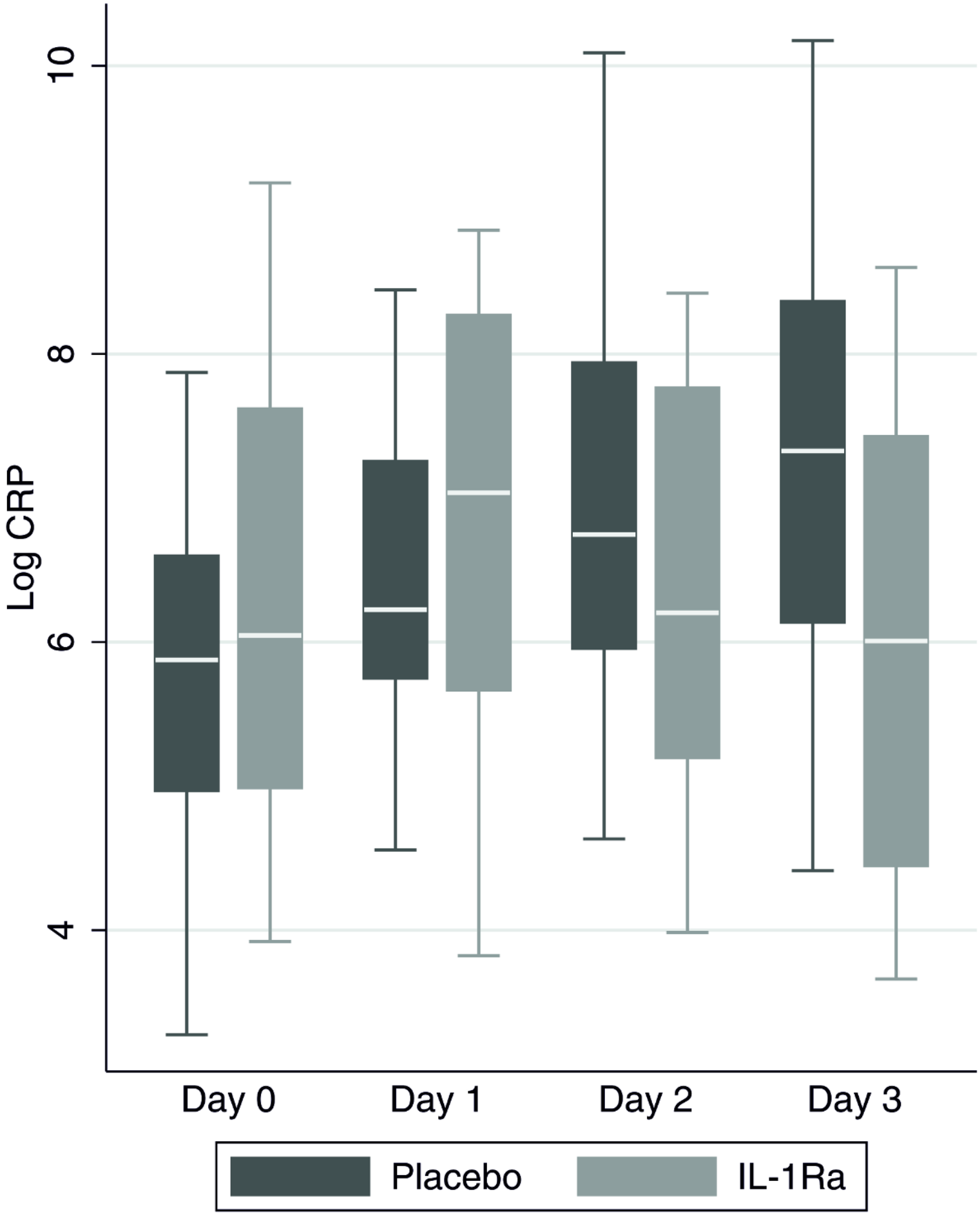
	Placebo (n=41)	IL-1Ra (n=39)
Mean (SD) age (y)	72 (13)	72 (12)
Male sex, n (%)	28 (68)	22 (56)
Pre-stroke mRS=0, n (%)	25 (61)	24 (65)
Median NIHSS (min, max)	12 (4 to 25)	12 (4 to 25)
IV alteplase, n (%)	31 (76)	27 (71)
Median (min, max) OTN time, (mins)	141 (8, 263)	168 (83, 267)
Median (min, max) DTN time, (mins)	53 (25, 120)	58 (19, 188)
IA thrombectomy, n (%)	2 (5)	0
Median (min, max) onset to initiation of test drug, (mins)	266 (220 to 322)	260 (225 to 282)
Median (min, max) doses of test drug received	6 (4 to 6)	6 (5 to 6)
Risk factors, n (%)		
Hypertension	22 (56)	13 (35)
Previous stroke or TIA	13 (32)	13 (34)
Atrial fibrillation	6 (16)	4 (11)
Coronary artery disease	14 (35)	10 (27)
Diabetes mellitus	6 (15)	8 (22)
Current smoker	10 (26)	5 (14)
Dyslipidemia	7 (18)	7 (18)

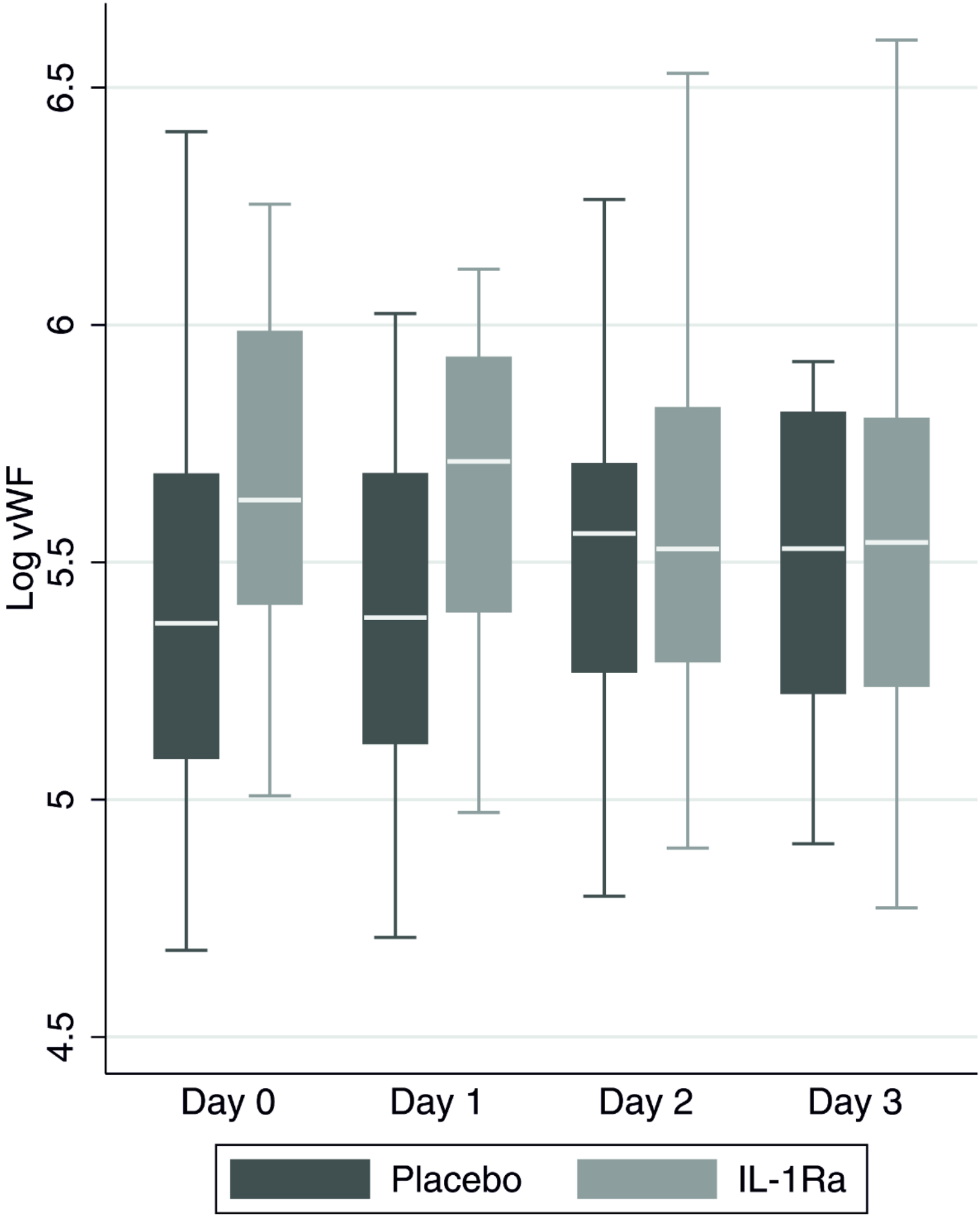
mRS indicates modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; IV, intravenous; OTN, onset to needle; DTN, door to needle; IA, intra-arterial; TIA, transient ischemic attack

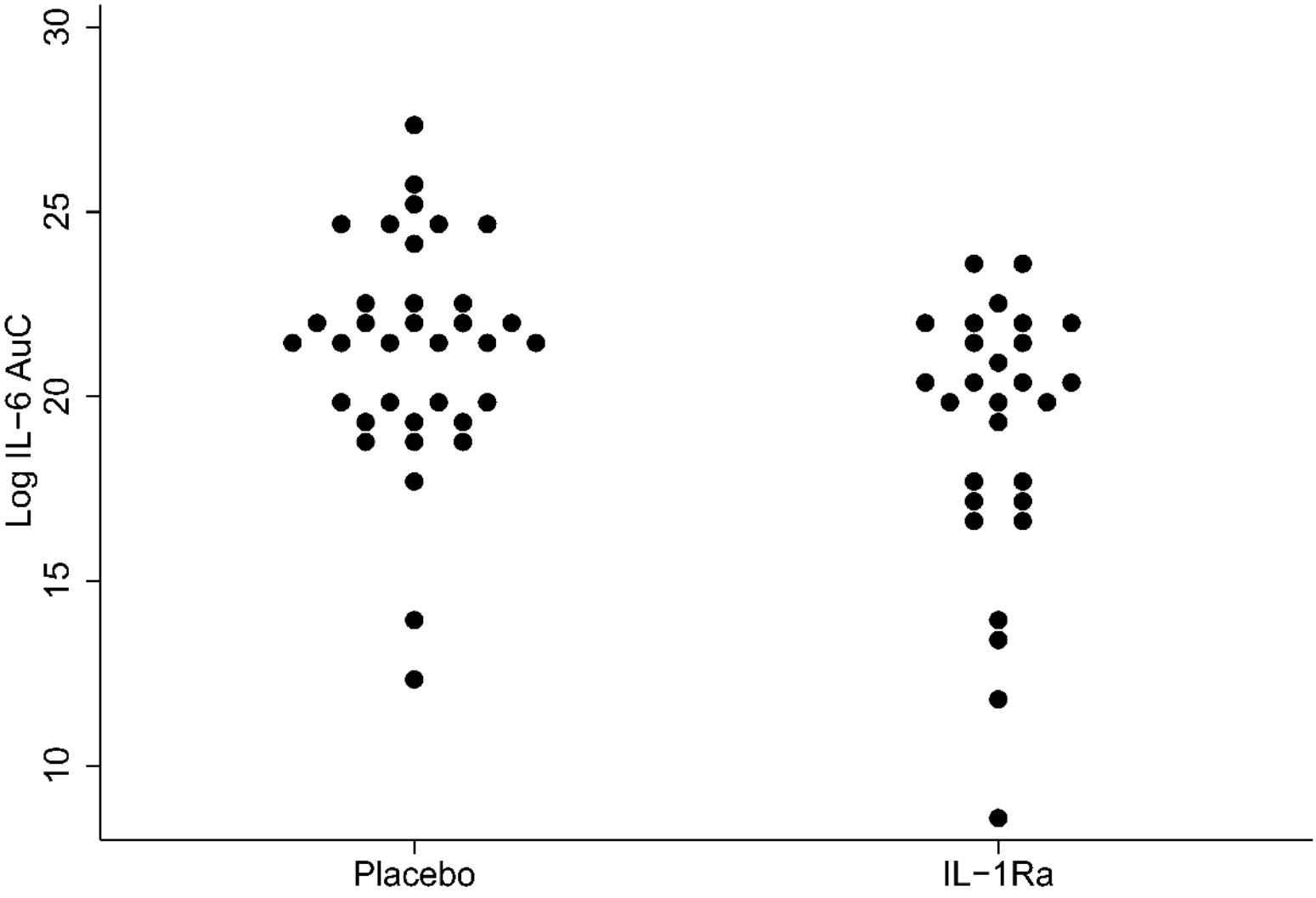
Figure 1

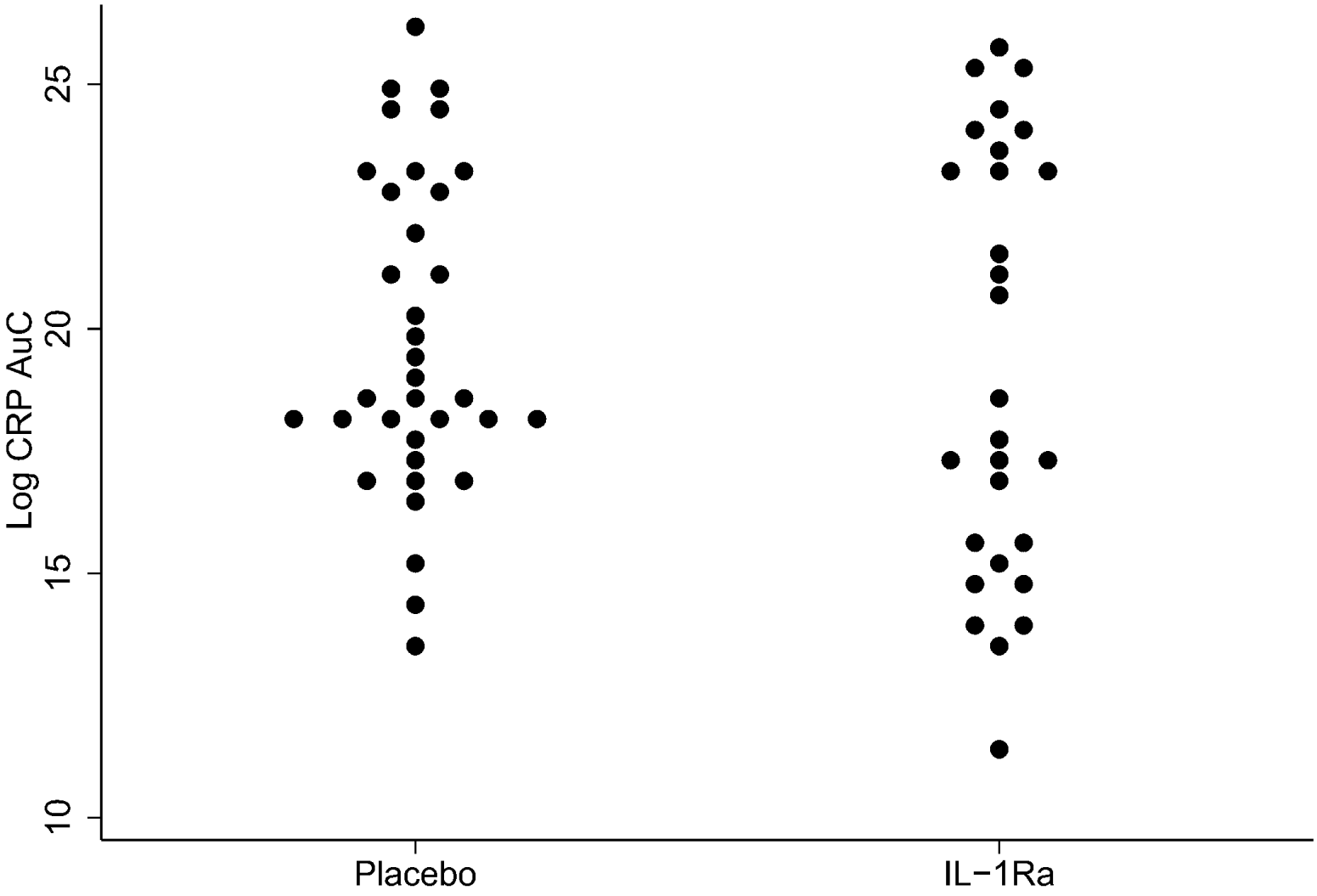

Screened (n=742)
Not eligible (n=535)
Eligible (n=207)
Presented 2100-0700 (n=89)
Approached (n=118)
Declined participation (n=38)
Randomised (n=80)
Placebo (n=41)
IL-1Ra (n=39)
Insufficient samples for primary outcome (n=6)
Repatriated before completion (n=2)
Insufficient samples for primary outcome (n=11)
Repatriated before completion (n=4)
Analysed (n=35)
Primary outcome (n=63)
Analysed (n=28)
3 month outcome data (n=39)
5 withdrawals
6 died
3 month outcome data (n=36)
2 withdrawals
5 died


Log IL−6
12
10
8
6
4
2
Day 0
Day 1
Day 2
Day 3
Placebo
IL−1Ra


Log CRP
10
8
6
4
Day 0
Day 1
Day 2
Day 3
Placebo
IL-1Ra


Log vWF
6.5
6
5.5
5
4.5
Day 0
Day 1
Day 2
Day 3
Placebo
IL-1Ra


Log IL-6 AuC
30
25
20
15
10
Placebo
IL-1Ra


Log CRP AuC
10
15
20
25
Placebo
IL−1Ra

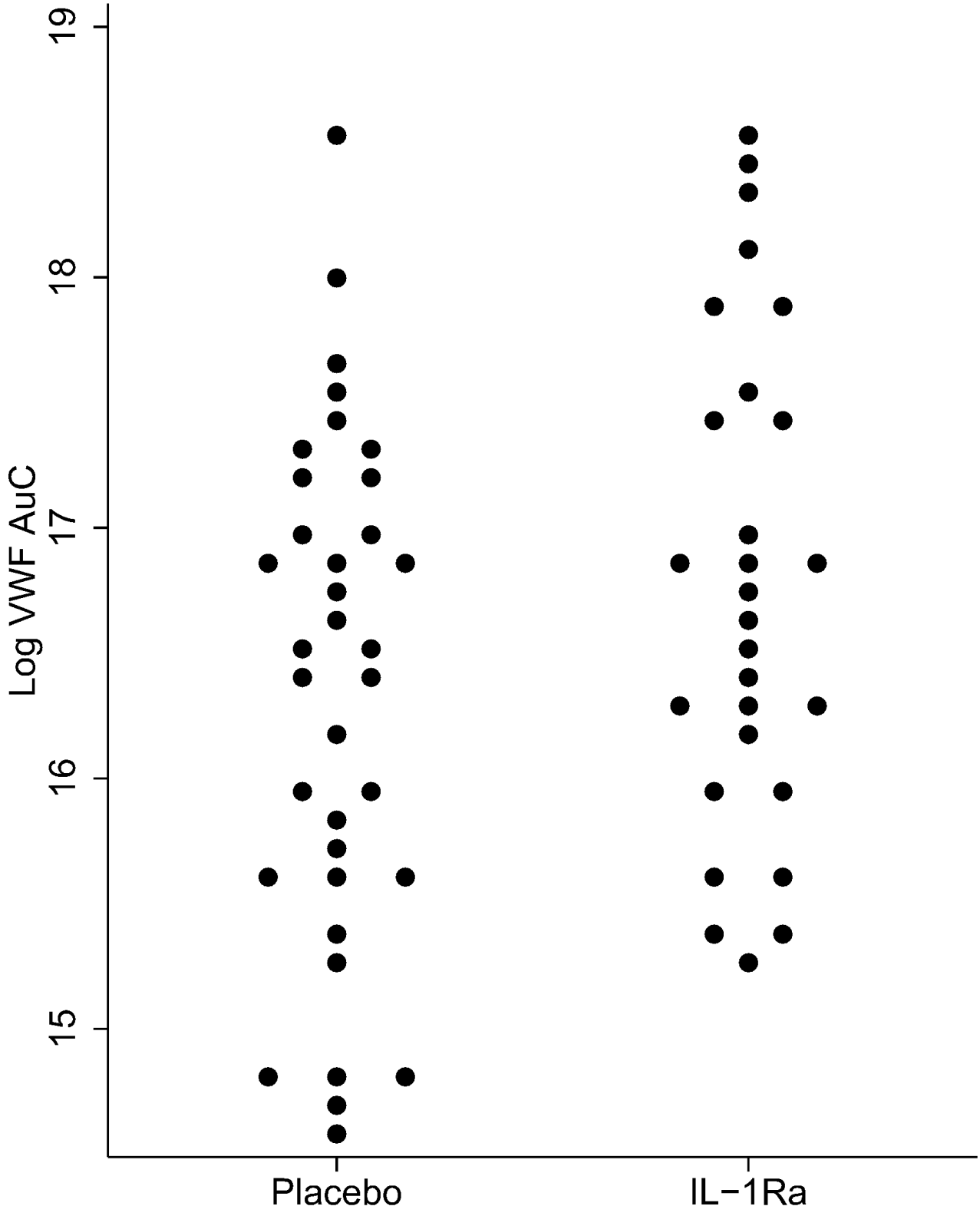
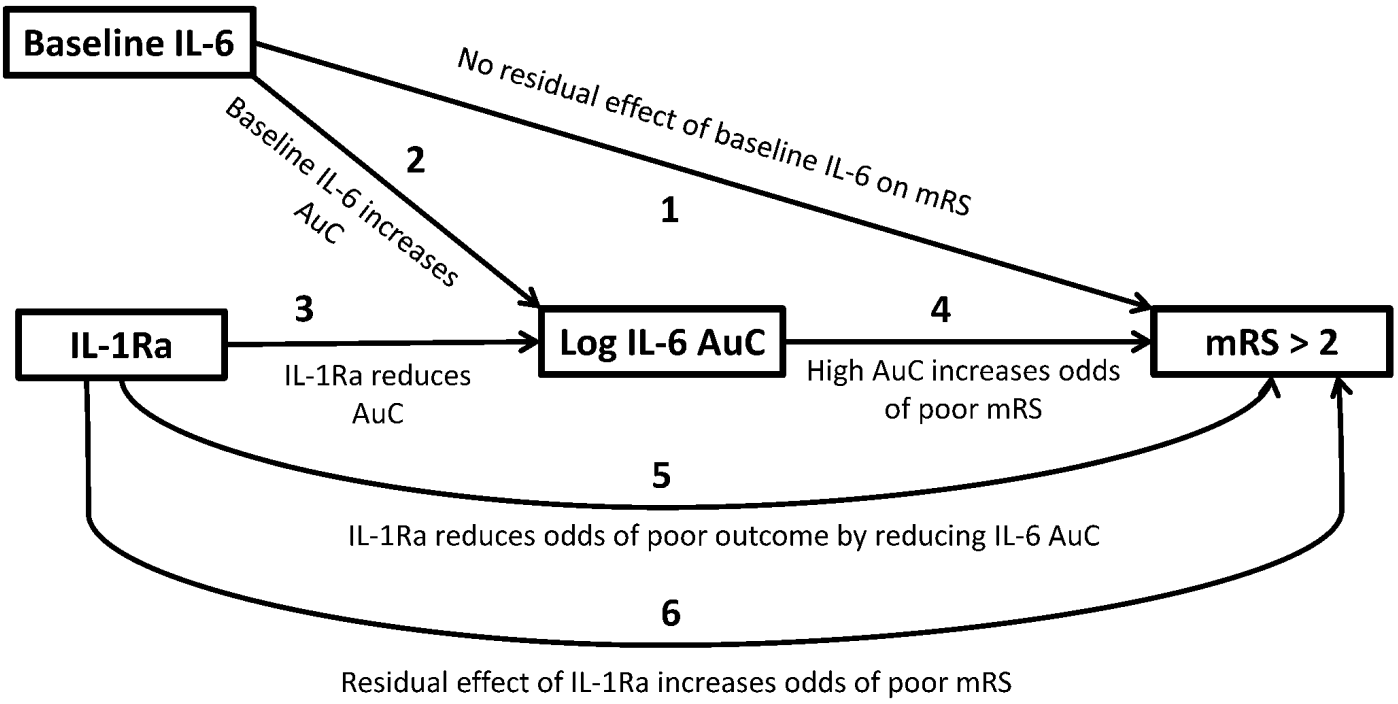

Log VWF AuC
19
18
17
16
15
Placebo
IL−1Ra

Figure 4



	Regression coefficient or OR (95% C.I)
1	**OR = 0.68 (0.37 to 1.24), p = 0.21**
2	**1.80 (1.34 to 2.27), p < 0.01**
3	**-2.65 (-3.86 to -1.44), p < 0.01**
4	**OR = 1.39 (1.06 to 1.82), p = 0.02**
5	**OR=0.42 (0.19 to 0.94), p = 0.04**
6	**OR = 4.58 (1.19 to 17.71), p = 0.03**

IL-6, interleukin-6; IL-1Ra, interleukin-1 receptor antagonist; AUC, area under the curve; mRS, modified Rankin Scale