Efficacy of UVC-treated, pathogen-reduced platelets *versus* untreated platelets: a randomized controlled non-inferiority trial

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Supplementary Appendix

Supplement to: Efficacy of UVC-treated, pathogen-reduced platelets versus untreated platelets: a randomized controlled non-inferiority trial

Principal investigators and study sites

Study site	Principal investigator	No. of included patients
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Department of Hematology and Oncology, University Hospital Frankfurt, Goethe University, Frankfurt/Main	Gesine Bug	30
Department of Hematology and Oncology, Municipal Hospital, Bremen	Ulrich Ritter	22
Department of Hematology and Oncology, University Hospital, Mannheim	Stefan Klein	16
Medical Clinic I, Department of Hematology and Oncology, University Hospital, Carl Gustav Carus Faculty of Medicine, Dresden	Nael Alakel	8
Department of Hematology and Oncology, Municipal Hospital, Osnabrück	Rudolf Peceny	5
Department of Hematology and Oncology, Municipal Hospital Frankfurt-Hoechst, Frankfurt/Main	Hans G. Derigs	4
Medical Clinic III, University Hospital, Ulm	Frank Stegelmann	4
Department of Hematology and Oncology, Municipal Hospital, Kassel	Martin Wolf	3
Institute for Transfusion Medicine, University Hospital Ulm, Ulm, Germany; and Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, German Red Cross Blood Service Baden-Württemberg - Hessia, Ulm	Hubert Schrezenmeier	2

Supplementary methods

Study design

The trial protocol was written by a steering committee and approved by both the German national authority (Paul-Ehrlich-Institute) and a central ethic committee. The study was conducted in compliance with the German Pharmaceutical Act (AMG) and with Good Clinical Practice according to the International Conference on Harmonization guidelines, and it met the ethical requirements of the Declaration of Helsinki. A Data Safety Monitoring Board (DSMB) supervised the study for safety aspects at regular intervals. The study was monitored for quality and regulatory compliance. The authors vouch for the integrity of the data and analyses reported. The study was sponsored by the German Red Cross Blood Service NSTOB. The study is registered in the German Clinical Trials Register (http://www.drks.de) under study number DRKS00011156.

Only the local blood transfusion service personnel responsible for processing and issuing the study platelets had knowledge of the patient's randomization arm.

Inclusion and exclusion criteria

Inclusion criteria required a life expectancy of more than eight weeks and an Eastern Cooperative Oncology Group (ECOG) status of 2 or less. Patients with documented refractoriness to platelet transfusions due to human leukocyte antigen (HLA) and/or human platelet antigen (HPA) antibodies, a history or current diagnosis of an autoimmune disease that affects hemostasis, a history or diagnosis of thrombotic thrombocytopenic purpura or hemolytic uremic syndrome, acute or chronic disseminated intravascular coagulation (DIC), active bleeding (grade 3 or higher according to the World Health Organization [WHO] bleeding scale)[1] requiring one or more RBC transfusions and/or therapeutic platelet transfusions at time of enrolment were excluded. Other main exclusion criteria were: acute promyelocytic leukemia (AML, FAB subtype M3), extensive splenomegaly (defined as a palpable spleen felt more than 4 cm below costal margin), history of severe anaphylactic transfusion reaction, and severe uncontrolled infection.

Stratification and randomization

The number of study transfusion episodes was limited to 8 because it has been shown that the count increment response decreases with multiple transfusions, even in non-alloimmunized patients.[2,3] After informed consent was obtained, eligible patients were registered and randomized to receive untreated PCs or UV-treated PCs using a centralized, web-based allocation tool, stratified by site and by whether or not the patient had received or was receiving allogeneic hematopoietic stem cell transplantation. The random allocation schedule was prepared using a 1:1 ratio and random permuted blocks with a block size of 4. Patients could be randomized only once and received a maximum of eight platelet transfusion episodes (see definition below).

Platelet products and transfusion policy

Reference and UVC-treated platelet products were either collected by apheresis (Trima Accel, TerumoBCT, and Amicus, Fresenius Kabi) or prepared from five buffy coats and resuspended in SSP^+ (Macopharma) platelet additive solution (PAS), which is equivalent to PAS-E.[4] All platelet units were leukoreduced and prepared in accordance with the manufacturer's specifications for UVC treatment (platelet concentration: 0.8×10^9/mL to 1.4×10^9/mL, platelet yield: 2.2×10^{11} to 5.25×10^{11}, residual plasma: 30% to 40%). All PCs were stored at 20-24°C for up to 5 days. A platelet transfusion episode was defined as one platelet transfusion or multiple platelet transfusions if the time between transfusions was not more than two hours. Off-protocol platelet transfusions (i.e., conventional platelet transfusions) were allowed during the treatment period if no study platelet units were available. Post-transfusion increments were not determined for off-protocol transfusions. ABO-identical PCs were generally used if available, but minor and major ABO-incompatible platelet transfusions were also allowed. Transfusion failure was defined as a 1-hour CCI of less than 7.5 and a 24-hour CCI of less than 4.5.[5] Clinical refractoriness was defined as the occurrence of transfusion failure of two

consecutive study platelet transfusions. Immunological refractoriness was defined as clinical refractoriness combined with the detection of platelet antibodies.

The recommended prophylactic platelet transfusion trigger threshold for patients with hematologic or oncologic diseases is 10,000/µL if clinical risk factors are absent. We generally used the recommended threshold, but other triggers (e.g. prior to interventions) could be used if medically indicated.[6]

Data collection

Platelet product information and patient data were recorded on a centrally processed electronic case report form managed by an independent clinical research organization (Alcedis, Gießen, Germany). To evaluate the risk of alloimmunization to UVC-irradiated platelets, samples were taken for platelet antibody testing prior to the first platelet transfusion, at the end of the safety follow-up period, and at any time when immunological refractoriness to platelet transfusion was suspected (Figure S1).

Adverse events and transfusion reactions

Data on adverse events and transfusion reactions were collected from the start of the first study transfusion through the first 15 days of the 30-day safety follow-up period using the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. During the following 15 days of the follow-up period, only signs and symptoms of TA-GvHD were assessed and documented. The severity of adverse events was scored on the basis of the most severe symptom or sign.

Power calculation and statistical analysis

Based on previous studies with 100% plasma PCs,[7-11] we performed sample size calculations for the UVC arm using a mean 1-hour CCI of 15.7 and a standard deviation of 7.0 and determined that 75 patients per arm were needed for a power of 95%, an alpha error of 0.025 and a 1:1 ratio. Assuming that the proportion of subjects who were non-compliant or lost to

follow-up would be 10%, it was estimated that a total of 166 patients were required (83 per arm).

Primary and secondary efficacy endpoint analyses were performed for both the intention-to-treat (ITT) and the per-protocol (PP) populations. An analysis of secondary safety endpoints was performed on the ITT population only. The ITT population included all randomized patients who received at least one platelet transfusion. The PP population is the ITT population subset including all randomized patients who met all inclusion criteria, did not meet any exclusion criteria, and did not receive the wrong type of platelet transfusion (i.e., platelet products not prepared according to treatment assignment) or any off-protocol platelet transfusions during the study period.

Depending on the data distribution, Student's t-test, Wilcoxon's rank sum-test, the Chi-2 test or Fisher's exact test were performed to compare categorical patient characteristics or ordinal or continuous characteristics by arm. All analyses were performed using the software package SAS Release 9.4 (9.4m3, STAT 14.1). P values <0.05 were considered significant. Analyses of CIs and CCIs were patient-based and accounted for the fact that patients had different numbers of platelet transfusions over variable periods of time, and that platelet transfusions given repeatedly to the same patient were not independent.

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Supplementary tables

Table S1. Off-protocol platelet transfusions (based on ITT)

		UVC	Control	p
Platelet transfusion episodes	n	316	245	0.804
Off-protocol*	n (%)	14 (4.43)	12 (4.90)	
Per-protocol	n (%)	302 (95.57)	233 (95.10)	
Percentage of off-protocol transfusions received per patient*				0.682
0%	n (%)	76 (87.36)	76 (90.48)	
1-25%	n (%)	7 (8.05)	4 (4.76)	
26-50%	n (%)	4 (4.60)	4 (4.76)	
Patients with one treatment error†	n (%)	2 (2.30)	3 (3.57)	0.678

*Off-protocol transfusion: transfusion of non-study (conventional) platelets
†Treatment error: patient received platelets from the wrong treatment group

Table S2. Adverse events and serious adverse events by severity and relationship to transfusion (based on ITT)

		UVC	Control	p
Adverse events	n	741	633	0.328
Patients with adverse events	n (%)	85 (97.70)	80 (95.24)	
Maximum grade per patient				0.163
Grade 1	n (%)	3 (3.53)	8 (10.00)	
Grade 2	n (%)	30 (35.29)	18 (22.50)	
Grade 3	n (%)	36 (42.35)	33 (41.25)	
Grade 4	n (%)	15 (17.65)	18 (22.50)	
Grade 5	n (%)	1 (1.18)	3 (3.75)	
Relationship to platelet transfusion*				
Excluded	n (%)	66 (75.86)	65 (77.38)	0.858
Unlikely	n (%)	60 (68.97)	49 (58.33)	0.156
Possible	n (%)	16 (18.39)	7 (8.33)	0.073
Likely, probable	n (%)	2 (2.30)	1 (1.19)	1.000
Certain	n (%)	0 (0.00)	1 (1.19)	0.491
Not assessable	n (%)	1 (1.15)	1 (1.19)	1.000
Serious adverse events	n	10	10	
Patients with serious adverse events	n (%)	10 (11.63)	8 (9.52)	
Maximum grade per patient				0.385
Grade 1	n (%)	0 (0.00)	1 (12.50)	
Grade 2	n (%)	1 (10.00)	0 (0.00)	
Grade 3	n (%)	6 (60.00)	3 (37.50)	
Grade 4	n (%)	2 (20.00)	1 (12.50)	
Grade 5	n (%)	1 (10.00)	3 (37.50)	
Relationship to platelet transfusion*				
Excluded	n (%)	3 (3.45)	0 (0.00)	0.858
Unlikely	n (%)	7 (8.05)	8 (9.52)	0.792
Possible	n (%)	0 (0.00)	0 (0.00)	
Likely, probable	n (%)	0 (0.00)	0 (0.00)	
Certain	n (%)	0 (0.00)	0 (0.00)	
Not assessable	n (%)	0 (0.00)	0 (0.00)	

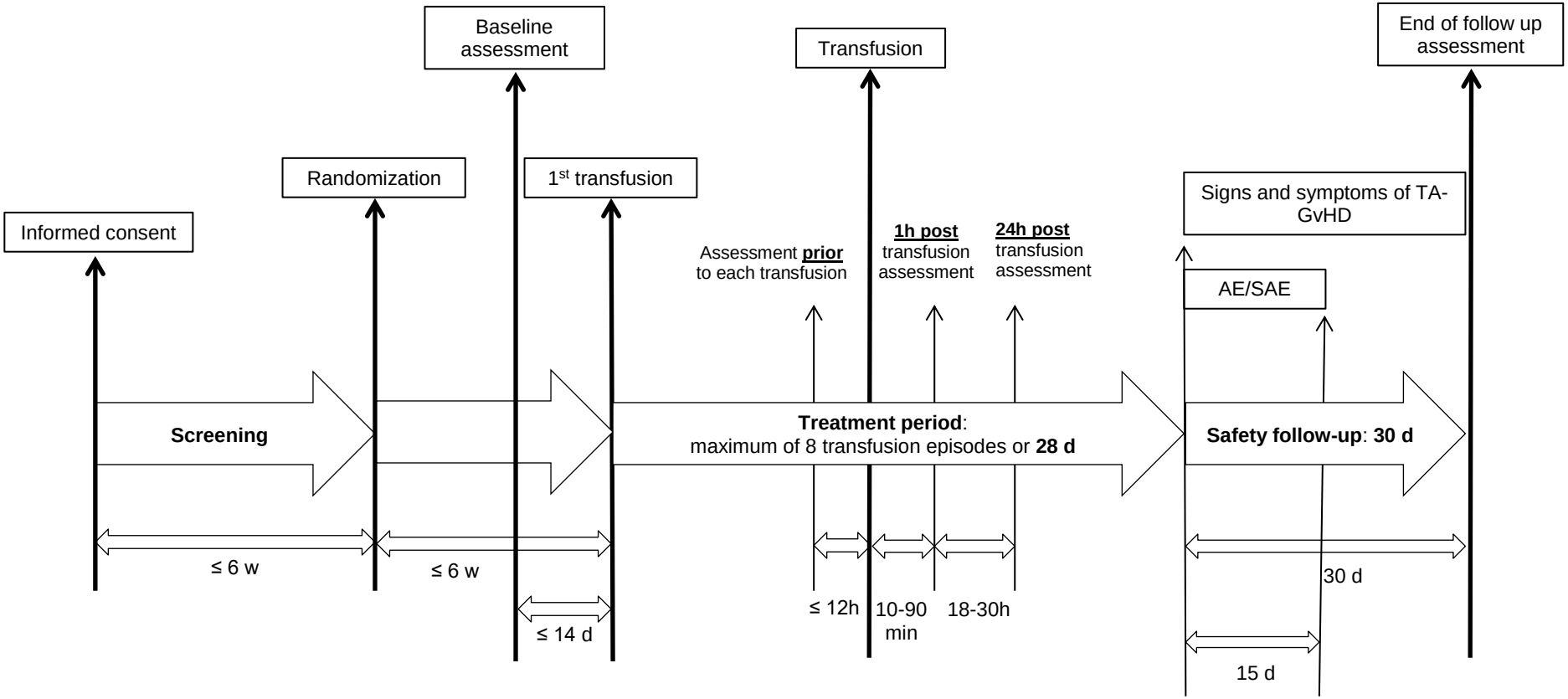
*Multiple answers possible

Table S3. Adverse events related to platelet transfusion and serious adverse events - signs and symptoms by system organ class (SOC) and preferred term (PT) (based on ITT)

		UVC	Control
Adverse event related to platelet transfusion	n (%)	34 (100.00)	11 (100.00)
Blood and lymphatic system disorders	n (%)	1 (2.94)	0 (0.00)
Febrile neutropenia	n (%)	1 (2.94)	0 (0.00)
General disorders and administration site conditions	n (%)	10 (29.41)	2 (18.18)
Chills	n (%)	4 (11.76)	1 (9.09)
Pyrexia	n (%)	6 (17.65)	1 (9.09)
Immune system disorders	n (%)	4 (11.76)	2 (18.18)
Hypersensitivity	n (%)	4 (11.76)	2 (18.18)
Infections and infestations	n (%)	1 (2.94)	0 (0.00)
Enterococcal infection	n (%)	1 (2.94)	0 (0.00)
Injury, poisoning and procedural complications	n (%)	8 (23.53)	4 (36.36)
Infusion-related reaction	n (%)	0 (0.00)	1 (9.09)
Refractoriness to platelet transfusion	n (%)	8 (23.53)	3 (27.27)
Investigations	n (%)	1 (2.94)	1 (9.09)
Platelet count decreased	n (%)	1 (2.94)	1 (9.09)
Respiratory, thoracic and mediastinal disorders	n (%)	2 (5.88)	0 (0.00)
Epistaxis	n (%)	2 (5.88)	0 (0.00)
Skin and subcutaneous tissue disorders	n (%)	7 (20.59)	1 (9.09)
Petechiae	n (%)	2 (5.88)	0 (0.00)
Rash	n (%)	4 (11.76)	1 (9.09)
Urticaria	n (%)	1 (2.94)	0 (0.00)
Vascular disorders	n (%)	0 (0.00)	1 (9.09)
Embolism	n (%)	0 (0.00)	1 (9.09)
Serious adverse events	n	10 (100.00)	10 (100.00)
Blood and lymphatic system disorders	n (%)	0 (0.00)	2 (20.00)
Febrile neutropenia	n (%)	0 (0.00)	2 (20.00)
Cardiac disorders	n (%)	2 (20.00)	2 (20.00)
Angina pectoris	n (%)	0 (0.00)	1 (10.00)
Atrial fibrillation	n (%)	0 (0.00)	1 (10.00)
Atrial tachycardia	n (%)	1 (10.00)	0 (0.00)
Cardiac arrest	n (%)	1 (10.00)	0 (0.00)
General disorders and administration site conditions	n (%)	1 (10.00)	1 (10.00)
Pyrexia	n (%)	1 (10.00)	1 (10.00)
Immune system disorders	n (%)	1 (10.00)	0 (0.00)
Graft versus host disease	n (%)	1 (10.00)	0 (0.00)
Infections and infestations	n (%)	6 (60.00)	5 (50.00)
Bronchitis	n (%)	1 (10.00)	0 (0.00)
Cytomegalovirus infection	n (%)	0 (0.00)	1 (10.00)
Device related infection	n (%)	1 (10.00)	0 (0.00)
Lung infection	n (%)	1 (10.00)	1 (10.00)
Pneumonia	n (%)	1 (10.00)	0 (0.00)
Sepsis	n (%)	0 (0.00)	1 (10.00)
Septic shock	n (%)	1 (10.00)	0 (0.00)
Soft tissue infection	n (%)	0 (0.00)	1 (10.00)
Staphylococcal infection	n (%)	1 (10.00)	0 (0.00)
Neoplasms, benign, malignant and unspecified	n (%)	0 (0.00)	1 (10.00)

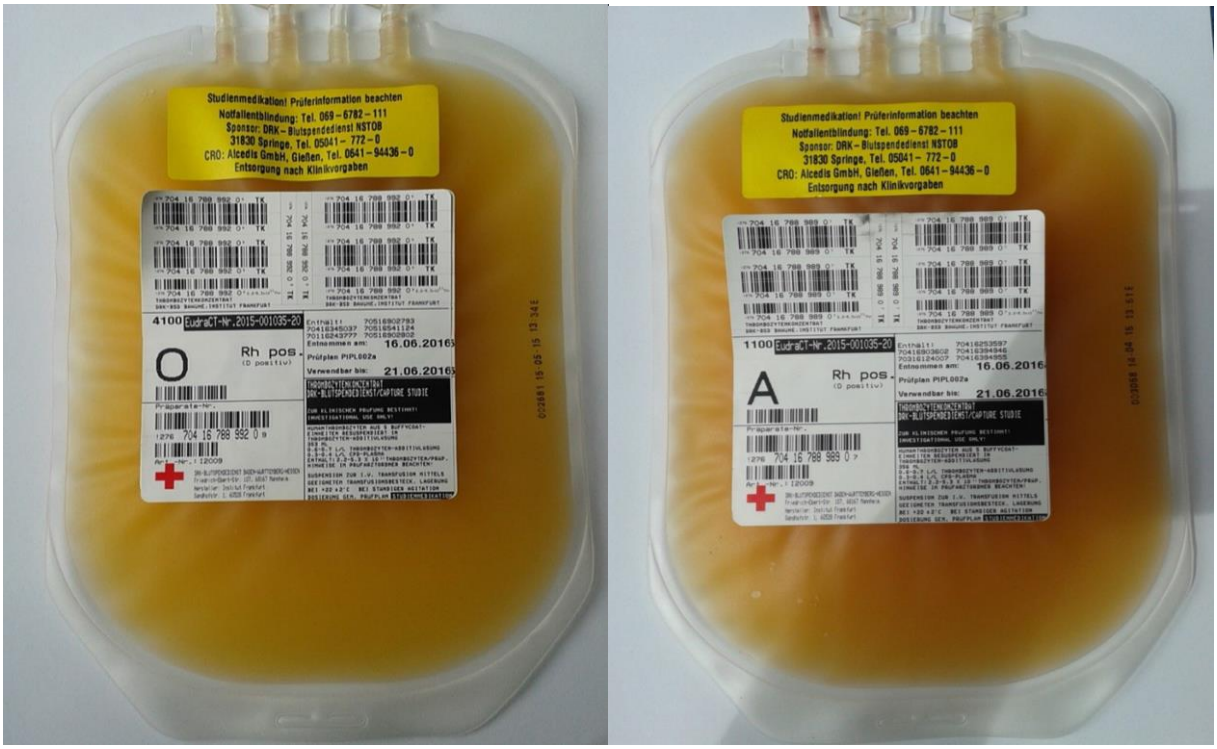
Supplementary figures

Figure S1. Study flow diagram



AE, adverse event; SAE, serious adverse event, TA-GvHD, transfusion-associated graft-versus-host disease

Figure S2. Photographic examples of the UVC-treated and control platelet units



UVC-treated Control

Photographic examples of a UVC-treated platelet unit (left) and a control platelet unit (right) are shown. Blood bags including labelling were identical for UVC-treated and control platelets, ensuring double blinding.

CONSORT Statement 2006 - Checklist for Non-inferiority and Equivalence Trials

Items to include when reporting a non-inferiority or equivalence randomized trial

PAPER SECTION **And topic**	**Item**	**Descriptor**	**Reported on Page #**
TITLE & ABSTRACT	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned"), s*pecifying that the trial is a non-inferiority or equivalence trial.*	1, 4
INTRODUCTION Background	2	Scientific background and explanation of rationale, *including the rationale for using a non-inferiority or equivalence design.*	5
METHODS Participants	3	Eligibility criteria for participants *(detailing whether participants in the non-inferiority or equivalence trial are similar to those in any trial(s) that established efficacy of the reference treatment)* and the settings and locations where the data were collected.	6; Supplement: 3-6
Interventions	4	Precise details of the interventions intended for each group *detailing whether the reference treatment in the non-inferiority or equivalence trial is identical (or very similar) to that in any trial(s) that established efficacy* and how and when they were actually administered.	6, Supplement: 4,5
Objectives	5	Specific objectives and hypotheses, *including the hypothesis concerning non-inferiority or equivalence*.	7
Outcomes	6	Clearly defined primary and secondary outcome measures *detailing whether the outcomes in the non-inferiority or equivalence trial are identical (or very similar) to those in any trial(s) that established efficacy of the reference treatment* and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors).	6,7
Sample size	7	How sample size was determined *detailing whether it was calculated using a non-inferiority or equivalence criterion and specifying the margin of equivalence with the rationale for its choice*. When applicable, explanation of any interim analyses and stopping rules (*and whether related to a non-inferiority or equivalence hypothesis*).	7, Supplement: 5,6
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence, including details of any restrictions (*e.g.*, blocking, stratification)	Supplement: 4
Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence (*e.g.*, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	Supplement: 4
Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	Supplement: 4
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.	6, Supplement: 3
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s), *specifying whether a one or two-sided confidence interval approach was used*. Methods for	7, Supplement: 5,6

		additional analyses, such as subgroup analyses and adjusted analyses.	
RESULTS Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	8, 26
Recruitment	14	Dates defining the periods of recruitment and follow-up.	8
Baseline data	15	Baseline demographic and clinical characteristics of each group.	8, 19
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was *"intention-to-treat" and/or alternative analyses were conducted*. State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	8
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (*e.g.*, 95% confidence interval). *For the outcome(s) for which non-inferiority or equivalence is hypothesized, a figure showing confidence intervals and margins of equivalence may be useful*.	9, 10, 21, 22, 27
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	-
Adverse events	19	All important adverse events or side effects in each intervention group.	10, 24; Supplement: 9,10
DISCUSSION Interpretation	20	Interpretation of the results, taking into account the *non-inferiority or equivalence hypothesis and any other* study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	11-15
Generalizability	21	Generalizability (external validity) of the trial findings.	14-15
Overall evidence	22	General interpretation of the results in the context of current evidence.	11-15