Clinical trial results:
A Single Blind Phase IV Pharmacodynamic Study to Evaluate Neutrophil Distribution Kinetics and Function Following Single-Dose Tocilizumab Treatment in Healthy Subjects.

Summary

EudraCT number	2013-002341-11
Trial protocol	GB
Global end of trial date	06 March 2015

Results information

Result version number	v1 (current)
This version publication date	26 February 2016
First version publication date	26 February 2016

Trial information

Trial identification

Sponsor protocol code	WA29049

Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	NCT01991990
WHO universal trial number (UTN)	-

Notes:

Sponsors

Sponsor organisation name	F. HoffmannLa Roche AG
Sponsor organisation address	Grenzacherstrasse 124, CH4070, Basel, Switzerland,
Public contact	Roche Trial Information Hotline, F. Hoffmann-La Roche Ltd, +41 61 6878333, global.trial_information@roche.com
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Notes:

Paediatric regulatory details

Is trial part of an agreed paediatric investigation plan (PIP)	No
Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?	No
Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Notes:

Results analysis stage

Analysis stage	Final
Date of interim/final analysis	06 March 2015
Is this the analysis of the primary completion data?	No

Global end of trial reached?	Yes
Global end of trial date	06 March 2015
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

This was a Phase IV, single-blind, two-arm study to explore the pharmacodynamics (PD) effects of tocilizumab (TCZ) on neutrophil redistribution, function, and survival in healthy participants, and to investigate differences between participants with greater than (>) 50% neutrophil count decrease at Day 4 relative to baseline and participants with less than or equal to (≤) 50% neutrophil count decrease at Day 4 relative to baseline.

Protection of trial subjects:

This study was conducted in full conformance with the International Conference on Harmonization (ICH) E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever afforded the greater protection to the individual. Studies conducted in the European Union/European Economic Area (EU/EEA) complied with the EU Clinical Trial Directive (2001/20/EC).

Background therapy: -

Evidence for comparator: -

Actual start date of recruitment	02 May 2014
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	No

Notes:

Population of trial subjects

Subjects enrolled per country

Country: Number of subjects enrolled	United Kingdom: 18
Worldwide total number of subjects	18
EEA total number of subjects	18

Notes:

Subjects enrolled per age group

In utero	0
Preterm newborn - gestational age < 37 wk	0
Newborns (0-27 days)	0
Infants and toddlers (28 days-23 months)	0
Children (2-11 years)	0
Adolescents (12-17 years)	0
Adults (18-64 years)	18

From 65 to 84 years	0
85 years and over	0

Subject disposition

Recruitment

Recruitment details:

There was a substantial protocol amendment for this study on 20Dec2014, post the Global End of Trial Date (GETD) of 10Dec2014. An error is shown: "The amendment date is not allowed. Amendment dates must not be later than the global end of the trial date". In order to resolve this error, the GETD is reported as 06Mar2015 (Final Database lock [DBL]).

Pre-assignment

Screening details:

The screening visit was up to 3 weeks before randomization to the first dose of study medication. Out of 23 screened participants; 5 participants discontinued (4=met exclusion criteria; 1=withdrew), 18 participants were included.

Period 1

Period 1 title	Overall study (overall period)
Is this the baseline period?	Yes
Allocation method	Randomised - controlled
Blinding used	Single blind
Roles blinded	Subject

Arms

Are arms mutually exclusive?	Yes
Arm title	Placebo

Arm description:

Participants received a single dose of placebo-matched to tocilizumab on Day 0.

Arm type	Placebo
Investigational medicinal product name	Placebo
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Infusion
Routes of administration	Intravenous use

Dosage and administration details:

Single dose of placebo injection was administered on Day 0

Arm title	Tocilizumab

Arm description:

Participants received a single dose of intravenous (IV) tocilizumab (TCZ) at a dose of 8 milligrams (mg) per kilogram (kg) body weight infusion over 1 hour on Day 0

Arm type	Experimental
Investigational medicinal product name	Tocilizumab
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Infusion
Routes of administration	Intravenous use

Dosage and administration details:

Participants received single IV infusion of tocilizumab based on their body weight on Day 0

Number of subjects in period 1	Placebo	Tocilizumab
Started	6	12
Completed	6	12

Baseline characteristics

Reporting groups	
Reporting group title	Placebo
Reporting group description:	
Participants received a single dose of placebo-matched to tocilizumab on Day 0.	
Reporting group title	Tocilizumab
Reporting group description:	
Participants received a single dose of intravenous (IV) tocilizumab (TCZ) at a dose of 8 milligrams (mg) per kilogram (kg) body weight infusion over 1 hour on Day 0	

Reporting group values	Placebo	Tocilizumab	Total
Number of subjects	6	12	18
Age categorical			
Units: Subjects			
Age continuous			
Units: years			
arithmetic mean	29.5	34.5	
standard deviation	± 11	± 12.6	-
Gender categorical			
Units: Subjects			
Female	0	0	0
Male	6	12	18

End points

End points reporting groups

Reporting group title	Placebo
Reporting group description:	
Participants received a single dose of placebo-matched to tocilizumab on Day 0.	
Reporting group title	Tocilizumab
Reporting group description:	
Participants received a single dose of intravenous (IV) tocilizumab (TCZ) at a dose of 8 milligrams (mg) per kilogram (kg) body weight infusion over 1 hour on Day 0	
Subject analysis set title	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)
Subject analysis set type	Sub-group analysis
Subject analysis set description:	
Participants who received a single dose of IV TCZ at a dose of 8 mg/kg body weight infusion over 1 hour on Day 0 and with ≤50% neutrophil count decrease at Day 4 relative to baseline were included in this group.	
Subject analysis set title	Tocilizumab (PMN-Low Group)
Subject analysis set type	Sub-group analysis
Subject analysis set description:	
Participants who received a single dose of IV TCZ at a dose of 8 mg/kg body weight infusion over 1 hour on Day 0 and with >50% neutrophil count decrease at Day 4 relative to baseline were included in this group.	

Primary: Neutrophil Redistribution Analysis on Day 4 (Neutrophil Nadir)

End point title	Neutrophil Redistribution Analysis on Day 4 (Neutrophil
End point description:	
On Day 4 participants had neutrophils isolated from 100 milliliters (mL) of acid-citrate dextrose (ACD)-anti-coagulated autologous blood and labeled in autologous plasma with up to 2.5 megaBecquerel (MBq) 111 Indium (111In)-tropolonate before being reinjected. Participants rested for 45 minutes (min) post-injection for neutrophil equilibrium between the circulating and marginating neutrophil pools. Whole-body profiling performed with 2 highly sensitive scintillation detectors with the recorded counts corrected for the physical decay of 111In to allow measurement of the effect of TCZ on the normal redistribution pattern of neutrophils and assessment of margination of neutrophils in the presence of TCZ. Distribution of radiolabelled neutrophils on Day 4 (45 min post re-injection) in the blood, expressed as percentages of total body counts (TBCs). Safety analysis population used for analysis of this endpoint.	
End point type	Primary
End point timeframe:	
Day 4	

Notes:

[1] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[2] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	4[3]	7	
Units: percentage of total body count				

arithmetic mean (standard error)				
Blood	26.7 (± 4.7)	26.5 (± 1.9)	30.8 (± 5)	
Liver/Spleen	50.4 (± 1.6)	55 (± 3.1)	52.4 (± 1.1)	
Pelvic marrow	12.6 (± 0.9)	11.7 (± 1)	10.8 (± 1)	

Notes:

[3] - 1 participant was excluded due to external contamination affecting profiling data.

Statistical analyses

No statistical analyses for this end point

Primary: Neutrophil Redistribution Analysis on Day 5

End point title	Neutrophil Redistribution Analysis on Day 5[4][5]

End point description:

On Day 4 participants had neutrophils isolated from 100 mL of ACD-anti-coagulated autologous venous blood and labeled with up to 2.5 MBq 111In-tropolonate before being reinjected. Participants rested for 45 min post-injection to allow for neutrophil equilibrium between the circulating and marginating neutrophil pools. Whole-body profiling was performed in a heavily shielded dedicated whole-body counter with 2 highly sensitive scintillation detectors with the recorded counts corrected for the physical decay of 111In to allow measurement of the effect of TCZ on the normal redistribution pattern of neutrophils and assessment of margination of neutrophils in the presence of TCZ. Distribution of radiolabelled neutrophils and peak counts, on Day 5 (24-hours post re-injection) in liver/spleen (center) and pelvic bone marrow (right) were decay corrected and expressed as percentages of Day 4 (45 minutes post re-injection). Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Day 5

Notes:

[4] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[5] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	4[6]	7	
Units: percentage of Day 4 counts				
arithmetic mean (standard error)				
Liver/spleen peak counts (Day 5)	91 (± 5.1)	90.3 (± 5)	105.2 (± 3.3)	
Pelvic peak counts (Day 5)	187.8 (± 14.1)	178.4 (± 18.9)	129.1 (± 7)	

Notes:

[6] - 1 participant was excluded due to external contamination affecting profiling data.

Statistical analyses

No statistical analyses for this end point

Primary: Neutrophil Redistribution Analysis on Day 10

End point title	Neutrophil Redistribution Analysis on Day 10[7][8]

End point description:

On Day 4 participants had neutrophils isolated from 100 mL of ACD-anti-coagulated autologous venous blood and labeled with up to 2.5 MBq 111In-tropolonate before being reinjected. Participants rested for 45 min post-injection to allow for neutrophil equilibrium between the circulating and marginating neutrophil pools. Whole-body profiling was performed in a heavily shielded dedicated whole-body counter with 2 highly sensitive scintillation detectors with the recorded counts corrected for the physical decay of 111In to allow measurement of the effect of TCZ on the normal redistribution pattern of neutrophils and assessment of margination of neutrophils in the presence of TCZ. Distribution of radiolabelled neutrophils and peak counts, on Day 10 (6 days post re-injection) in liver/spleen (center) and pelvic bone marrow (right) were decay corrected and expressed as percentages of Day 4 (45 minutes post re-injection). Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Day 10

Notes:

[7] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[8] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	4[9]	7	
Units: percentage of Day 4 counts				
arithmetic mean (standard error)				
Liver/spleen peak counts (Day 10)	84.1 (± 6.2)	76.6 (± 3.3)	96.2 (± 2.9)	
Pelvic peak counts (Day 10)	180.3 (± 12.9)	175.6 (± 14.4)	132.6 (± 5)	

Notes:

[9] - 1 participant was excluded due to external contamination affecting profiling data.

Statistical analyses

No statistical analyses for this end point

Primary: Neutrophil Phagocytosis: Change From Baseline to Nadir (Day 4) in the Percentage of eFluor670-Positive (eFluoro670+) Neutrophils

End point title	Neutrophil Phagocytosis: Change From Baseline to Nadir (Day 4) in the Percentage of eFluor670-Positive (eFluoro670+) Neutrophils[10][11]

End point description:

Neutrophil phagocytosis was assessed by flow cytometry using heat-killed Staphylococcal pneumonia (S.pneumonia) bacteria labeled with eFluor670. Phagocytosis was quantified by measuring the eFluor670 fluorescence from neutrophils containing phagocytosed bacteria. Experiments were performed using neutrophils (PMN) only, PMN plus S. pneumonia at 4 degrees(°) centigrade (C) (to control for non-specific bacterial adherence to PMN cell surface), and PMN plus S. pneumonia at 37°C. Change from baseline in the percentage of eFluor670+ neutrophils was calculated on Day 4. Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Baseline, Day 4

Notes:

[10] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[11] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	5	7	
Units: percentage of eFlouro+ neutrophils				
arithmetic mean (standard error)				
PMN only	0 (± 0)	0 (± 0)	0 (± 0)	
PMN and S.pneumonia at 4°C	-1 (± 1)	2 (± 1)	5 (± 2)	
PMN and S.pneumonia at 37°C	4 (± 2)	7.5 (± 2)	9 (± 2)	

Statistical analyses

No statistical analyses for this end point

Primary: Neutrophil Phagocytosis: Change From Baseline to Nadir (Day 4) in Median Fluorescence Intensity (MFI) of eFluor670+ Neutrophils

End point title	Neutrophil Phagocytosis: Change From Baseline to Nadir (Day 4) in Median Fluorescence Intensity (MFI) of eFluor670+ Neutrophils[12][13]

End point description:

Neutrophil phagocytosis was assessed by flow cytometry using heat-killed Staphylococcal pneumonia bacteria labeled with eFluor670. Phagocytosis was quantified by measuring the eFluor670 fluorescence from neutrophils containing phagocytosed bacteria. Experiments were performed using neutrophils (PMN) only, PMN plus S. pneumonia at 4°C (to control for non-specific bacterial adherence to PMN cell surface), and PMN plus S. pneumonia at 37°C. Change from baseline in the eFluor670+ MFI was calculated on Day 4. Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Baseline, Day 4

Notes:

[12] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[13] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	5	7	
Units: median fluoresence intensity				
arithmetic mean (standard error)				
PMN only	-1 (± 3)	2 (± 7)	2 (± 2)	
PMN and S.pneumonia at 4°C	-2 (± 3)	39 (± 18)	32 (± 7)	
PMN and S.pneumonia at 37°C	685 (± 443)	979 (± 350)	810 (± 217)	

Statistical analyses

No statistical analyses for this end point

Primary: Neutrophil Respiratory Burst: Change From Baseline to Nadir (Day 4) in the Production of Reactive Oxygen Species as Measured by Chemiluminescence (Relative Light Units - Absolute)

End point title	Neutrophil Respiratory Burst: Change From Baseline to Nadir (Day 4) in the Production of Reactive Oxygen Species as Measured by Chemiluminescence (Relative Light Units - Absolute)[14][15]

End point description:

Neutrophils generate a respiratory burst using reactive oxygen species (ROS) to kill invading pathogens. When luminol is used as a substrate for ROS, a chemical reaction is produced resulting in photon emission (chemiluminescence) in primed and unprimed neutrophils following formyl-methionyl-leucyl-phenylalanine (fMLP) stimulation which is quantifiable. fMLP stimulation of the respiratory burst is mediated through activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in primed neutrophils. The maximal fMLP response is observed in primed neutrophils and is an ex vivo measure of the capacity of neutrophils to respond to pathogenic stimuli. In the current experiments, neutrophils were primed with tumor necrosis factor alpha (TNFα). Light emission was recorded on a luminometer. Absolute change from baseline in the production of ROS on Day 4 was reported. Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Baseline, Day 4

Notes:

[14] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[15] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	5	7	
Units: relative light units				
arithmetic mean (standard error)				

Unprimed neutrophils	-16710 (± 12346)	-5553 (± 6409)	-2766 (± 3291)	
TNFα primed neutrophils	82465 (± 16727)	-45257 (± 17540)	64072 (± 16130)	

Statistical analyses

No statistical analyses for this end point

Primary: Neutrophil Survival: Change From Baseline to the Nadir (Day 4) in the Percentage of Apoptotic Neutrophils as Measured by Microscopic Morphololgy

End point title	Neutrophil Survival: Change From Baseline to the Nadir (Day 4) in the Percentage of Apoptotic Neutrophils as Measured by Microscopic Morphololgy[16][17]

End point description:

Neutrophil apoptosis was measured using microscopy method with slides stained with Diff-Quik (modified Wright Giemsa stain) and morphology examined under oil immersion light microscopy with 100 times magnification. Neutrophils constitutively undergo apoptosis when cultured ex vivo, and this can be delayed by the addition of agents such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or TNFα. Apoptotic neutrophils were characterized with dark and pyknotic nuclei compared to the viable neutrophils. Change from baseline in the percentage of apoptotic neutrophils on Day 4 measured by microscopy is reported. Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Baseline, Day 4

Notes:

[16] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[17] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	5		
Units: percentage of apoptotic neutrophils				
arithmetic mean (standard error)				
Untreated neutrophils	-6 (± 3)	-1.8 (± 4)	-6 (± 5)	
GM-CSF neutrophils	-1 (± 1)	-7 (± 5)	-12 (± 6)	
TNFα neutrophils	-2 (± 4)	3 (± 4)	-4 (± 5)	

Statistical analyses

No statistical analyses for this end point

Primary: Neutrophil Survival: Change From Baseline to the Nadir in the Percentage of Apoptotic Neutrophils as Measured by Flow Cytometry

End point title	Neutrophil Survival: Change From Baseline to the Nadir in the Percentage of Apoptotic Neutrophils as Measured by Flow Cytometry[18][19]

End point description:

Ageing neutrophils translocate phosphatidylserine from the inner leaflet of the plasma membrane to the outer leaflet during the early stages of apoptosis. This translocation can be measured due to the affinity of Annexin V (AV) to bind exposed phosphatidylserine. Propidium Iodide (PI) is normally membrane-impermeable but enters cells in late apoptosis when their plasma membrane becomes leaky. Neutrophils constitutively undergo apoptosis when cultured ex vivo, and this can be delayed by the addition of agents such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or TNFα. Apoptosis was assessed by flow cytometry with fluorescein isocyanate-labeled recombinant human AV (AV-FITC) and PI staining and the change from baseline in the percentage of apoptotic neutrophils on Day 4 measured by flow cytometry is reported. Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Baseline, Day 4

Notes:

[18] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[19] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	5	7	
Units: percentage of apoptotic neutrophils				
arithmetic mean (standard error)				
Untreated neutrophils	-5 (± 2)	-8 (± 4)	-7 (± 5)	
GM-CSF neutrophils	-4 (± 2)	-13 (± 4)	-9 (± 8)	
TNFα neutrophils	-3 (± 4)	3 (± 2)	3 (± 5)	

Statistical analyses

No statistical analyses for this end point

Primary: Neutrophil Morphology: Change From Baseline to Nadir in the Number of Neutrophils With Shape Change Measured Using Flow Cytometry

End point title	Neutrophil Morphology: Change From Baseline to Nadir in the Number of Neutrophils With Shape Change Measured Using Flow Cytometry[20][21]

End point description:

Neutrophil shape change is an indicator of the chemotactic ability of neutrophils to respond to and migrate to sites of inflammation. For determination of neutrophil shape change, fresh (0 min control), phosphate-buffered saline (PBS) control (30 min control) and formyl-methionyl-leucyl-phenylalanine (fMLP)-stimuated (30 min fMLP) PMNs (at 5 × 10^6 PMNs/ milliliter [mL]) were fixed with CellFIX, 90 microliters (µL) transferred to each sample tube, and cold PBS added to stop further reaction. Shape

change was assessed by measuring forward scatter (FSC) on flow cytometry. Change from baseline in the number of neutrophils with shape change on Day 4 was reported. Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Baseline, Day 4

Notes:

[20] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[21] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	5	7	
Units: neutrophils with shape change				
arithmetic mean (standard error)				
0 min control	1613 (± 842)	-359 (± 2613)	4790 (± 1341)	
30 min control	4882 (± 3069)	-2309 (± 4439)	8814 (± 3016)	
30 min fMLP	-1889 (± 2800)	-1667 (± 4093)	1524 (± 3515)	

Statistical analyses

No statistical analyses for this end point

Primary: Neutrophil Morphology: Change From Baseline to the Nadir (Day 4) in the Percentage of Neutrophils With Shape Change Measured by Flow Cytometry (FSC-High Cells)

End point title	Neutrophil Morphology: Change From Baseline to the Nadir (Day 4) in the Percentage of Neutrophils With Shape Change Measured by Flow Cytometry (FSC-High Cells)[22][23]

End point description:

Neutrophil shape change is an indicator of the chemotactic ability of neutrophils to respond to and migrate to sites of inflammation. For determination of neutrophil shape change, fresh (0 min control), PBS control (30 min control) and fMLP-stimuated (30 min fMLP) PMNs (at 5 × 10^6 PMNs/ mL) were fixed with CellFIX, 90 µL transferred to each sample tube, and cold PBS added to stop further reaction. Shape change was assessed by measuring FSC on flow cytometry. Change from baseline in the percentage of neutrophils with shape change on Day 4 was reported. Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Baseline, Day 4

Notes:

[22] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[23] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	5	7	
Units: percentage of shape changed neutrophils				
arithmetic mean (standard error)				
0 min control	14 (± 11)	0 (± 3)	2 (± 2)	
30 min control	17 (± 8)	1 (± 4)	6 (± 3)	
30 min fMLP	1 (± 3)	0 (± 2)	-1 (± 3)	

Statistical analyses

No statistical analyses for this end point

Primary: Neutrophil Morphology: Change From Baseline to the Nadir (Day 4) in the Percentage of Neutrophils With Shape Change Measured by Microscopic Morphology

End point title	Neutrophil Morphology: Change From Baseline to the Nadir (Day 4) in the Percentage of Neutrophils With Shape Change Measured by Microscopic Morphology[24][25]

End point description:

Neutrophil shape change is an indicator of the chemotactic ability of neutrophils to respond to and migrate to sites of inflammation. For determination of neutrophil shape change, fresh (0 min control), PBS control (30 min control) and fMLP-stimuated (30 min fMLP) PMNs (at 5 × 10^6 PMNs/ mL) were fixed with CellFIX, 90 µL transferred to each sample tube, and cold PBS added to stop further reaction. Shape change was assessed by microscopy with neutrophils classified as shape-changed if they contained > 1 cell surface bleb or irregularity and change from baseline in percentage of neutrophil with shape change on Day 4 was reported. Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Baseline, Day 4

Notes:

[24] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[25] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	5	7	
Units: percentage of shape changed neutrophils				
arithmetic mean (standard error)				
0 min control	12 (± 10)	-3 (± 4)	0 (± 2)	
30 min control	13 (± 11)	-2 (± 4)	-1 (± 1)	
30 min fMLP	2 (± 6)	4 (± 5)	-1 (± 3)	

Statistical analyses

No statistical analyses for this end point

Primary: Absolute Median Fluorescence Intensities of Neutrophil Adhesion Molecules

End point title	Absolute Median Fluorescence Intensities of Neutrophil Adhesion Molecules[26][27]

End point description:

Neutrophil surface receptor expression may be used to characterize the activation status of neutrophils. Fresh (0 min), PBS control (30 min) and fMLP-stimulated (30 min) PMNs (5 × 10^6 PMNs/mL) were fixed with CellFIX, and 90 µL transferred to each tube containing antibody mixture (2 µL cluster of differentiation [CD] 11b-brilliant violet (BV) 421, 2 µL CD16-FITC, 5 µL CD62L-allophycocyanin (APC) and 5 µL CD162-phycoerythrin [PE]) or isotype control mixture of equivalent volumes. After 30 minutes of incubation on ice and in the dark, cold PBS was added to stop further reaction. Surface marker expressions were quantified by flow cytometry. Safety analysis population used for analysis of this endpoint.

End point type	Primary

End point timeframe:

Day 4

Notes:

[26] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: No statistical analysis was planned for this study.

[27] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Tocilizumab arm was split in to two groups, "PMN-High Group" and "PMN-Low Group" and the results are reported for these 2 groups.

End point values	Placebo	Tocilizumab (Polymorphonuclear Leukocyte [PMN])-High Group)	Tocilizumab (PMN-Low Group)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	6	5	7	
Units: medianfluorescence intensity				
arithmetic mean (standard error)				
CD11b - 0 min control	12633 (± 2062)	15751 (± 2043)	17764 (± 1910)	

CD11b - 30 min control	13144 (± 2416)	16047 (± 2058)	18134 (± 1836)	
CD11b - 30 min fMLP	39629 (± 2699)	44481 (± 5049)	41339 (± 2723)	
CD16 - 0 min control	15822 (± 741)	16872 (± 1874)	15901 (± 1476)	
CD16 - 30 min control	13771 (± 706)	14885 (± 1744)	13475 (± 1269)	
CD16 - 30 min fMLP	17145 (± 884)	18229 (± 2613)	14707 (± 2205)	
CD62L - 0 min control	563 (± 37)	650 (± 201)	767 (± 65)	
CD62L - 30 min control	493 (± 38)	588 (± 181)	615 (± 37)	
CD62L - 30 min fMLP	6 (± 3)	5 (± 9)	9 (± 12)	
CD162 - 0 min control	3393 (± 454)	3253 (± 895)	3849 (± 314)	
CD162 - 30 min control	3088 (± 433)	2972 (± 820)	3355 (± 347)	
CD162 - 30 min fMLP	1571 (± 219)	1568 (± 472)	1733 (± 154)	

Statistical analyses

No statistical analyses for this end point

Adverse events

Adverse events information	
Timeframe for reporting adverse events:	
From baseline to 8 weeks of safety followup (approximately 8 months)	
Assessment type	Non-systematic

Dictionary used	
Dictionary name	MedDRA
Dictionary version	17.1

Reporting groups	
Reporting group title	Placebo
Reporting group description:	
Participants received a single dose of placebo-matched to tocilizumab on Day 0.	
Reporting group title	Tocilizumab
Reporting group description:	
Participants received a single dose of intravenous (IV) tocilizumab (TCZ) at a dose of 8 milligrams (mg) per kilogram (kg) body weight infusion over 1 hour on Day 0	

Serious adverse events	Placebo	Tocilizumab	
Total subjects affected by serious adverse events			
subjects affected / exposed	0 / 6 (0.00%)	0 / 12 (0.00%)	
number of deaths (all causes)	0	0	
number of deaths resulting from adverse events			

Frequency threshold for reporting non-serious adverse events: 0 %

Non-serious adverse events	Placebo	Tocilizumab	
Total subjects affected by non-serious adverse events			
subjects affected / exposed	3 / 6 (50.00%)	5 / 12 (41.67%)	
Investigations			
Alanine aminotransferase increased			
subjects affected / exposed	0 / 6 (0.00%)	1 / 12 (8.33%)	
occurrences (all)	0	1	
Aspartate aminotransferase increased			
subjects affected / exposed	0 / 6 (0.00%)	2 / 12 (16.67%)	
occurrences (all)	0	2	
Blood bilirubin increased			

subjects affected / exposed occurrences (all) Eosinophil count increased subjects affected / exposed occurrences (all)	0 / 6 (0.00%) 0 1 / 6 (16.67%) 1	1 / 12 (8.33%) 1 0 / 12 (0.00%) 0	
Nervous system disorders Headache subjects affected / exposed occurrences (all)	2 / 6 (33.33%) 2	1 / 12 (8.33%) 1	
Gastrointestinal disorders Nausea subjects affected / exposed occurrences (all)	0 / 6 (0.00%) 0	1 / 12 (8.33%) 1	
Respiratory, thoracic and mediastinal disorders Oropharyngeal pain subjects affected / exposed occurrences (all)	0 / 6 (0.00%) 0	2 / 12 (16.67%) 2	
Skin and subcutaneous tissue disorders Rash erythematous subjects affected / exposed occurrences (all)	0 / 6 (0.00%) 0	1 / 12 (8.33%) 1	

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
17 December 2013	The protocol was amended to add a 24-hour window to the Day 10 study visit and clarify that the neutrophil killing assay was not considered a key outcome of the study.
07 April 2014	The protocol amendment corrected the TCZ dosing information for participants weighing more than 100 kg and aligned the maximum TCZ dose of 800 mg with the current TCZ Summary of Product Characteristics (SmPC). Weight cutoffs for participants ≤ 50 kg and > 50 kg to ≤ 100 kg were modified and a new weight category of > 50 kg to ≤ 75 kg was added.
20 December 2014	The protocol was amended to change the grouping of participants from grading according to Common Terminology Criteria (CTC) Grade (Grade 1 or 2 vs. Grade 3 or 4) neutrophil count to grouping by a relative reduction in absolute neutrophil counts of ≤ 50% or > 50% at the time of the expected neutrophil nadir in relation to baseline counts because of the lower than expected rate of CTC Grade 3 or 4 neutrophil counts. Preliminary review of the data indicated this would allow for analysis of neutrophil distribution in TCZ-treated participants while eliminating unnecessary exposure of participants to TCZ and 111In. Although this protocol amendment was decided prior to actual GETD (10Dec2014), formal agreement was received afterwards.

Notes:

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

Limitations of the trial such as small numbers of subjects analysed or technical problems leading to unreliable data.

The actual GETD was 10Dec2014. To resolve the error ("The amendment date is not allowed. Amendment dates must not be later than the global end of the trial date") related to protocol amendment on 20Dec2014, GETD is reported as 06Mar2015 (Final DBL).

Notes: