



Clinical trial results:

Developmental Clinical Sciences: Does GM-CSF restore effective neutrophil function in critically ill patients?

Summary

EudraCT number	2011-005815-10
Trial protocol	GB
Global end of trial date	12 February 2015

Results information

Result version number	v1 (current)
This version publication date	11 August 2016
First version publication date	11 August 2016

Trial information

Trial identification

Sponsor protocol code	AJSEB001
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Additional study identifiers

ISRCTN number	ISRCTN95325384
ClinicalTrials.gov id (NCT number)	NCT01653665
WHO universal trial number (UTN)	-
Other trial identifiers	UKCRN ID: 12337, REC Ref: 12/YH/0083, IRAS ID: 91653

Notes:

Sponsors

Sponsor organisation name	Newcastle Upon Tyne Hospitals NHS Foundation Trust
Sponsor organisation address	Freeman Hospital, Freeman Rd, Newcastle upon Tyne, United Kingdom, NE7 7DN
Public contact	Professor John Simpson,, Newcastle Upon Tyne Hospitals NHS Foundation Trust, 44 01912087770, j.simpson@ncl.ac.uk
Scientific contact	Professor John Simpson,, Newcastle Upon Tyne Hospitals NHS Foundation Trust, 44 01912087770, j.simpson@ncl.ac.uk

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 October 2015
Is this the analysis of the primary completion data?	Yes
Primary completion date	12 February 2015
Global end of trial reached?	Yes
Global end of trial date	12 February 2015
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

Will GM-CSF (a drug used to stimulate white blood cells) be able to restore the ingestion of germs by neutrophils (the most important white blood cells in fighting bacterial and fungal infection) to fight off infection in critically ill patients on intensive care?

Protection of trial subjects:

Participants were treated in routine critical care setting. All patients enrolled in the study received daily monitoring.

Background therapy:

Patients received background care in keeping with their critical illness.

Evidence for comparator:

In controlled trials, the incidences of renal and hepatic dysfunction were comparable between Leukine and placebo treated patients. Patients who took part in the RCT component of the received either, an injection of the drug (GM-CSF) or an injection of a solution, with no effect (placebo or dummy drug). We then compared whether those patients who received the GM-CSF injection had an improvement in the function of their neutrophils compared to those who did not.

Actual start date of recruitment	17 September 2013
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	Yes

Notes:

Population of trial subjects

Subjects enrolled per country

Country: Number of subjects enrolled	United Kingdom: 38
Worldwide total number of subjects	38
EEA total number of subjects	38

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)	15
From 65 to 84 years	21
85 years and over	2

Subject disposition

Recruitment

Recruitment details:

The incapacitating nature of the condition precluded obtaining prospective informed consent from nearly all participants. Informed consent was sought from patients or a Personal or Professional Legal Representative. Due to the time dependent factors, a time limit of up to 24 hours to make a decision was given, but within 6 hrs if possible.

Pre-assignment

Screening details:

Daily screening of patients was performed on ICU (within 72hrs of admission). After consent, a blood sample was taken for assessment of neutrophil phagocytosis. If a patient's phagocytosis index was >50% the patient was not recruited, ie not all patients consented were included in the dose-finding study or RCT.

Pre-assignment period milestones

Number of subjects started	3634 ^[1]
Intermediate milestone: Number of subjects	screening completed: 3634
Intermediate milestone: Number of subjects	assessed for eligibility: 926
Intermediate milestone: Number of subjects	consented: 64
Intermediate milestone: Number of subjects	Eligibility confirmed by phagocytosis: 38
Number of subjects completed	38

Pre-assignment subject non-completion reasons

Reason: Number of subjects	screen fail: 3596
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Notes:

[1] - The number of subjects reported to have started the pre-assignment period are not the same as the worldwide number enrolled in the trial. It is expected that these numbers will be the same.

Justification: The total number of patients screened in ICUs was 3634. Of these only 38 were randomised into the trial and therefore 3596 patients were ineligible at various points during the screening process. Consent was obtained for 64 patients, however 26 of these were not eligible on the basis of phagocytosis screening following consent.

Period 1

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

Blinding implementation details:

Randomisation was a 1:1 ratio, with stratification by site, using a web-based randomisation service in NCTU. The randomised allocation schedule was generated by a statistician with no other involvement in the study to ensure independence and concealment of allocation. Permuted blocks of variable length were used to reduce the risk of breach of concealment of allocation. A treatment number was generated for each participant that links to the corresponding allocated study drug/placebo.

Arms

Are arms mutually exclusive?	Yes
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Arm title	GMCSF
Arm description: Each participant was randomly allocated to receive either study drug or placebo. As a placebo controlled, single-blind trial, patients, clinicians and the PIs were blinded to each patient's allocation. All trial drugs, whether GM-CSF or placebo, were packaged identically at the point of administration and identified only by a unique trial identifier.	
Arm type	Experimental
Investigational medicinal product name	Leukine
Investigational medicinal product code	LO3AA09
Other name	Sargramostim, Granulocyte-macrophage colony-stimulating factor, GM-CSF
Pharmaceutical forms	Solution for injection
Routes of administration	Subcutaneous use

Dosage and administration details:

Each patient randomised to the GMCSF arm received up to 4 days of treatment with GM-CSF (sargramostim, Leukine®) as a subcutaneous injection. GM-CSF (Sargramostim, Leukine®, 250 microgram/vial) dosed on actual body weight (3mcg/kg) up to a maximum dose of 450mcg/volume 1.8ml. The dose/volume administered was prescribed according to the weight ranges given to give the dose to the nearest 5kg.

Arm title	Placebo
Arm description: Each participant was randomly allocated to receive either study drug or placebo. As a placebo controlled, single-blind trial, patients, clinicians and the PIs were blinded to each patient's allocation. All trial drugs, whether GM-CSF or placebo, were packaged identically at the point of administration and identified only by a unique trial identifier.	
Arm type	Placebo
Investigational medicinal product name	Placebo
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Solution for injection
Routes of administration	Subcutaneous use

Dosage and administration details:

Each patient randomised to the placebo arm received up to 4 days of treatment with either 0.9% sodium chloride as a subcutaneous injection. 0.9% sodium chloride (placebo, 5ml /ampoule) dosed on actual body weight (3mcg/kg) up to a maximum dose of 450mcg/volume 1.8ml. The dose/volume administered was prescribed according to the weight ranges given to give the dose to the nearest 5kg.

Number of subjects in period 1	GMCSF	Placebo
Started	17	21
Eligibility confirmed by phagocytosis	17	21
Primary endpoint met	13 ^[2]	20 ^[3]
Day 9 data collected	11 ^[4]	17 ^[5]
Day 30 data collected	17	21
Completed	17	21

Notes:

[2] - The number of subjects at this milestone seems inconsistent with the number of subjects in the arm. It is expected that the number of subjects will be greater than, or equal to the number that completed, minus those who left.

Justification: milestones relate to the number randomised, and those for whom primary endpoint data was collected at Day 2, and then further data collected at Day 9 and Day 30. Some data was not collected at Days 9, however data was collected at Day 30 for all participants. Primary endpoint data would be missing if, for example, a patient died before day 2.

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Justification: milestones relate to the number randomised, and those for whom primary endpoint data was collected at Day 2, and then further data collected at Day 9 and Day 30. Some data was not collected at Days 9, however data was collected at Day 30 for all participants. Primary endpoint data would be missing if, for example, a patient died before day 2.

Baseline characteristics

Reporting groups

Reporting group title	GMCSF
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Reporting group description:

Each participant was randomly allocated to receive either study drug or placebo. As a placebo controlled, single-blind trial, patients, clinicians and the PIs were blinded to each patient's allocation. All trial drugs, whether GM-CSF or placebo, were packaged identically at the point of administration and identified only by a unique trial identifier.

Reporting group title	Placebo
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Reporting group description:

Each participant was randomly allocated to receive either study drug or placebo. As a placebo controlled, single-blind trial, patients, clinicians and the PIs were blinded to each patient's allocation. All trial drugs, whether GM-CSF or placebo, were packaged identically at the point of administration and identified only by a unique trial identifier.

Reporting group values	GMCSF	Placebo	Total
Number of subjects	17	21	38
Age categorical			
Units: Subjects			
In utero	0	0	0
Preterm newborn infants (gestational age < 37 wks)	0	0	0
Newborns (0-27 days)	0	0	0
Infants and toddlers (28 days-23 months)	0	0	0
Children (2-11 years)	0	0	0
Adolescents (12-17 years)	0	0	0
Adults (18-64 years)	7	8	15
From 65-84 years	8	13	21
85 years and over	2	0	2
Age continuous			
Units: years			
arithmetic mean	65.2	63.4	
standard deviation	± 16.7	± 15.7	-
Gender categorical			
Units: Subjects			
Female	7	6	13
Male	10	15	25
Case mix of patients in GRIP trial. Circumstances that led to them being recruited into the trial			
Circumstances that led to patients being recruited into the trial.			
Units: Subjects			
Elective medical	0	0	0
Elective surgical	2	2	4
Emergency medical	14	15	29
Emergency surgical	1	4	5
Weight			
Patients weight at baseline			
Units: kg			
arithmetic mean	72.6	83	

standard deviation	± 15.6	± 23.9	-
Length of stay in ICU			
Time in days that the patients stayed in the intensive care unit			
Units: days			
arithmetic mean	15.6	14.9	
standard deviation	± 10.4	± 10.1	-
Number of days of mechanical ventilation			
Number of days of mechanical ventilation			
Units: days			
arithmetic mean	10.9	10.3	
standard deviation	± 10.7	± 10.4	-
Baseline Neutrophil phagocytic capacity			
baseline measure of Neutrophil phagocytic capacity			
Units: percentage of neutrophils ingesting ≥ 2			
arithmetic mean	45.08	40.14	
standard deviation	± 4.59	± 8.21	-
Baseline reactive oxygen species (ROS) generation –primed cells			
Baseline measures of reactive oxygen species (ROS) generation –primed cells			
Units: nmoles of superoxide			
arithmetic mean	1.49	1.84	
standard deviation	± 1.36	± 1.65	-
Baseline reactive oxygen species (ROS) generation –second stimulus			
Baseline measures of reactive oxygen species (ROS) generation –second stimulus			
Units: nmoles per superoxide			
arithmetic mean	1.98	1.93	
standard deviation	± 1.53	± 1.77	-
baseline distance migrated on chemotaxis assay			
baseline measures of distance migrated on chemotaxis assay			
Units: micrometers			
arithmetic mean	418.3	404.4	
standard deviation	± 303.4	± 399.9	-
Baseline Early apoptosis - Apoptotic rate			
Baseline measures for Early apoptosis - Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria)			
Units: percentage of cells			
arithmetic mean	16.14	16.85	
standard deviation	± 13.26	± 12.45	-
baseline late apoptosis - Apoptotic rate			
late apoptosis - Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria)			
Units: percentage of cells			
arithmetic mean	11.98	7.42	
standard deviation	± 10.78	± 5.39	-
Baseline Sequential monocyte HLA-DR expression (QTB results)			
Baseline measures of Sequential monocyte HLA-DR expression (QTB results)			
Units: antibodies per cell			
arithmetic mean	6178.7	6381.9	
standard deviation	± 4145.8	± 5149.3	-

Baseline CD88 - (relative median fluorescence)			
Baseline measure CD88 - (relative median fluorescence)			
Units: ratio			
arithmetic mean	4.33	4.13	
standard deviation	± 3.93	± 2.47	-
Baseline percentage of T cells (naïve/memory t reg cells) CD45RO+RA-			
Baseline measure of percentage of T cells (naïve/memory t reg cells) CD45RO+RA-			
Units: percentage			
arithmetic mean	48.15	48.76	
standard deviation	± 12.7	± 15.35	-
Baseline Percentage of T cells (naïve/memory t reg cells) CD45RA+RO-			
Baseline measure of Percentage of T cells (naïve/memory t reg cells) CD45RA+RO-			
Units: percentage			
arithmetic mean	36.49	35.26	
standard deviation	± 12.84	± 12.82	-
Baseline Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-6			
Baseline measure of Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-6			
Units: pg/ml			
arithmetic mean	256.5	4017.6	
standard deviation	± 276.3	± 14641.3	-
baseline Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-8			
Baseline measure of Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-8			
Units: pg/ml			
arithmetic mean	147.78	516.04	
standard deviation	± 131.75	± 1096.31	-
Baseline Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-1β			
Baseline measure of Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-1β			
Units: pg/ml			
arithmetic mean	11.86	7.74	
standard deviation	± 15.66	± 12.02	-
Baseline Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-10			
Baseline measure of Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-10			
Units: pg/ml			
arithmetic mean	15.22	128.75	
standard deviation	± 13.59	± 483.82	-
Baseline Cytokine data measure of pro/anti-inflammatory mediators in serum - TNF			
Baseline measure of Cytokine data measure of pro/anti-inflammatory mediators in serum - TNF			
Units: pg/ml			
arithmetic mean	6.74	7.53	
standard deviation	± 9.99	± 13.71	-
Baseline Cytokine data measure of			

pro/anti-inflammatory mediators in serum -IL-12 p70			
Baseline measures of Cytokine data measure of pro/anti-inflammatory mediators in serum -IL-12 p70			
Units: pg/ml			
arithmetic mean	3.52	4.81	
standard deviation	± 6.39	± 9.85	-
Apache II score at admission			
Apache II score at admission			
Units: score			
median	19.5	21	
inter-quartile range (Q1-Q3)	16 to 27.5	18 to 23	-
Baseline sequential organ failure assessment (SOFA)			
Baseline sequential organ failure assessment (SOFA)			
Units: count			
median	9	8	
inter-quartile range (Q1-Q3)	4 to 10.5	6 to 10	-
Baseline ratio of lowest PaO2 to FiO2			
Baseline ratio of lowest PaO2 to FiO2			
Units: ratio			
arithmetic mean	23	27.4	
standard deviation	± 11	± 13.2	-
Baseline ratio of highest PaO2 to FiO2			
Baseline ratio of highest PaO2 to FiO2			
Units: ratio			
arithmetic mean	33.9	38.3	
standard deviation	± 14.9	± 17.7	-

End points

End points reporting groups

Reporting group title	GMCSF
Reporting group description: Each participant was randomly allocated to receive either study drug or placebo. As a placebo controlled, single-blind trial, patients, clinicians and the PIs were blinded to each patient's allocation. All trial drugs, whether GM-CSF or placebo, were packaged identically at the point of administration and identified only by a unique trial identifier.	
Reporting group title	Placebo
Reporting group description: Each participant was randomly allocated to receive either study drug or placebo. As a placebo controlled, single-blind trial, patients, clinicians and the PIs were blinded to each patient's allocation. All trial drugs, whether GM-CSF or placebo, were packaged identically at the point of administration and identified only by a unique trial identifier.	

Primary: Neutrophil phagocytic capacity 2 days after administration of GM-CSF/placebo

End point title	Neutrophil phagocytic capacity 2 days after administration of GM-CSF/placebo
End point description: Biological measure. Neutrophil phagocytic capacity 2 days after administration of GM-CSF/placebo as measured by the percentage of neutrophils ingesting ≥ 2 zymosan particles ex vivo	
End point type	Primary
End point timeframe: 2 days after baseline measure and administration of treatment	

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	21		
Units: percentage of neutrophils ingesting ≥ 2				
arithmetic mean (standard deviation)	57.21 ($\pm$ 13.17)	49.77 ($\pm$ 13.41)		

Statistical analyses

Statistical analysis title	Unadjusted difference phagocytic capacity at 2days
Statistical analysis description: Primary outcome measure - Neutrophil phagocytic capacity 2 days after administration of GM-CSF/placebo. We used the 2 sample t test to assess difference between the neutrophil phagocytic capacity 2 days after administration of GM-CSF/placebo by arm	
Comparison groups	GMCSF v Placebo

Number of subjects included in analysis	36
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.1075 ^[1]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	7.44
Confidence interval	
level	95 %
sides	2-sided
lower limit	-1.7
upper limit	16.58
Variability estimate	Standard error of the mean
Dispersion value	4.5

Notes:

[1] - Accept the null hypothesis and conclude no differences in the mean neutrophil phagocytic capacity between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates

Statistical analysis title	Adjusted difference phagocytic capacity after 2day
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Statistical analysis description:

Primary outcome measure - Neutrophil phagocytic capacity 2 days after administration of GM-CSF/placebo.

Difference in mean neutrophil phagocytic capacity at day 2 adjusted for the effects of site and baseline neutrophil phagocytic capacity

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	36
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.7295 ^[2]
Method	ANCOVA

Notes:

[2] - Adjusted for the effects of site and baseline neutrophil phagocytic capacity, there is no significance difference in mean neutrophil phagocytic capacity at day 2 between the 2 arms

Secondary: Neutrophil phagocytic capacity 4/5 days after administration of GM-CSF/placebo

End point title	Neutrophil phagocytic capacity 4/5 days after administration of GM-CSF/placebo
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End point description:

Biological measure. Neutrophil phagocytic capacity 4/5 days after administration of GM-CSF/placebo (as measured by the percentage of neutrophils ingesting ≥ 2 zymosan particles ex vivo).

End point type	Secondary
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End point timeframe:

4/5 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	12	16		
Units: percentage				
arithmetic mean (standard deviation)	62.28 ($\pm$ 15.69)	50.34 ($\pm$ 14.29)		

Statistical analyses

Statistical analysis title	Unadjusted difference phagocyte capacity 4/5days
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Statistical analysis description:

Secondary outcome measure - Neutrophil phagocytic capacity 4/5 days after administration of GM-CSF/placebo.

We used the 2 sample t test to assess difference between the neutrophil phagocytic capacity 4/5 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	28
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.0456 ^[3]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	11.95
Confidence interval	
level	95 %
sides	2-sided
lower limit	0.25
upper limit	23.64
Variability estimate	Standard error of the mean
Dispersion value	5.69

Notes:

[3] - Reject the null hypothesis and conclude that there are significant differences in the mean neutrophil phagocytic capacity between arms 4/5 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted difference phagocyte capacity 4/5days
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Statistical analysis description:

Secondary outcome measure - Neutrophil phagocytic capacity 4/5 days after administration of GM-CSF/placebo.

Difference in mean neutrophil phagocytic capacity at day 4/5 adjusted for the effects of site and baseline neutrophil phagocytic capacity

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	28
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.1512 ^[4]
Method	ANCOVA

Notes:

[4] - Adjusted for the effects of site and baseline neutrophil phagocytic capacity, there is no significance difference in mean neutrophil phagocytic capacity at day 4/5). No site effect (p=0.0956) or baseline dependence (p=0.0505)

Secondary: Neutrophil phagocytic capacity 6/7 days after administration of GM-

CSF/placebo

End point title	Neutrophil phagocytic capacity 6/7 days after administration of GM-CSF/placebo
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End point description:

Neutrophil phagocytic capacity 6/7 days after administration of GM-CSF/placebo (as measured by the percentage of neutrophils ingesting ≥ 2 zymosan particles ex vivo).

End point type	Secondary
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End point timeframe:

6/7 days after administration of GM-CSF/placebo

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	10	16		
Units: percentage of neutrophils ingesting ≥ 2				
arithmetic mean (standard deviation)	64.03 ($\pm$ 11.36)	52.66 ($\pm$ 15.01)		

Statistical analyses

Statistical analysis title	Unadjusted difference phagocytic capacity 6/7days
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Statistical analysis description:

Secondary outcome measure - Neutrophil phagocytic capacity 6/7 days after administration of GM-CSF/placebo.

We used the 2 sample t test to assess difference between the neutrophil phagocytic capacity 6/7 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	26
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.0513 ^[5]
Method	t-test, 2-sided
Parameter estimate	Mean difference (net)
Point estimate	11.37
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.07
upper limit	22.82
Variability estimate	Standard error of the mean
Dispersion value	5.54

Notes:

[5] - Accept the null hypothesis and conclude no differences in the mean neutrophil phagocytic capacity between arms 6/7 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted difference phagocytic capacity 6/7days
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Statistical analysis description:

Secondary outcome measure - Neutrophil phagocytic capacity 6/7 days after administration of GM-CSF/placebo

Difference in mean neutrophil phagocytic capacity at day 6/7 adjusted for the effects of site and baseline neutrophil phagocytic capacity

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	26
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.1575 ^[6]
Method	ANCOVA

Notes:

[6] - Adjusted for the effects of site and baseline neutrophil phagocytic capacity, there is no significance difference in mean neutrophil phagocytic capacity at day 6/7 (P=0.1575). No site effect (p=0.4833) or Baseline dependence (p=0.3120)

Secondary: Neutrophil phagocytic capacity 8/9 days after administration of GM-CSF/placebo

End point title	Neutrophil phagocytic capacity 8/9 days after administration of GM-CSF/placebo
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End point description:

Biological measure. Neutrophil phagocytic capacity 8/9 days after administration of GM-CSF/placebo (as measured by the percentage of neutrophils ingesting ≥ 2 zymosan particles ex vivo).

End point type	Secondary
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End point timeframe:

8/9 days after administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	9	11		
Units: percentage of neutrophils ingesting ≥ 2				
arithmetic mean (standard deviation)	68.33 ($\pm$ 9.12)	57.22 ($\pm$ 16.64)		

Statistical analyses

Statistical analysis title	Unadjusted difference phagocytic capacity 8/9days
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Statistical analysis description:

Secondary outcome measure - Neutrophil phagocytic capacity 8/9 days after administration of GM-CSF/placebo.

We used the 2 sample t test to assess difference between the neutrophil phagocytic capacity 8/9 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	20
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.09 ^[7]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	11.12

Confidence interval	
level	95 %
sides	2-sided
lower limit	-1.93
upper limit	24.16
Variability estimate	Standard error of the mean
Dispersion value	6.21

Notes:

[7] - Accept the null hypothesis and conclude no differences in the mean neutrophil phagocytic capacity between arms 8/9 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted difference phagocytic capacity 8/9days
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Statistical analysis description:

Secondary outcome measure - Neutrophil phagocytic capacity 8/9 days after administration of GM-CSF/placebo.

Difference in mean neutrophil phagocytic capacity at day 8/9 adjusted for the effects of site and baseline neutrophil phagocytic capacity

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	20
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.2452 [8]
Method	ANCOVA

Notes:

[8] - Adjusted for the effects of site and baseline neutrophil phagocytic capacity, there is no significance difference in mean neutrophil phagocytic capacity at day 8/9 (P=0.2452). No site effect (p=0.9648) or Baseline dependence (p=0.8611)

Secondary: Percentage of patients <50% Neutrophil phagocytic capacity at day 2

End point title	Percentage of patients <50% Neutrophil phagocytic capacity at day 2
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End point description:

Number of patients with less than 50% Neutrophil phagocytic capacity at day 2

End point type	Secondary
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End point timeframe:

2 days after administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	21		
Units: number with <50% Neutrophil capacity	3	12		

Statistical analyses

Statistical analysis title	Percentage of patients <50% and >=50% at day 2
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Statistical analysis description:

Secondary outcome measure - Neutrophil phagocytic capacity: Percentage of patients <50% and >=50% at day 2

Difference between GMCSF and placebo at day 2 for all patients (at 50% split)

Comparison groups	Placebo v GMCSF
Number of subjects included in analysis	36
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.041 ^[9]
Method	Fishers exact test
Parameter estimate	difference in proportions

Notes:

[9] - Reject the null hypothesis and conclude that there are differences in proportion of patients having neutrophil phagocytic capacity < 50% in either arm at day 2.

Secondary: Neutrophil capacity measured as 'area under curve' (AUC) up to day 8/9

End point title	Neutrophil capacity measured as 'area under curve' (AUC) up to day 8/9
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End point description:

Area under curve is calculated by summing the areas between each time point. This procedure is known as the linear trapezoidal rule.

In cases where there is missing data with no more data either before or after that point we do not calculate the area and treat that patient's area under curve as missing.

Neutrophil capacity measured as 'area under curve' (AUC) up to day 8/9

End point type	Secondary
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End point timeframe:

From day 0 to day 9 after administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	9	11		
Units: cells x 10 ⁹ /l x days				
arithmetic mean (standard deviation)	553.9 (± 73.5)	451.9 (± 85.2)		

Statistical analyses

Statistical analysis title	Unadjusted difference phagocyte by Area under curve
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Statistical analysis description:

Secondary outcome measure - Neutrophil capacity measured as 'area under curve' (AUC) up to day 8/9. We used the 2 sample t test to assess difference between 'area under the curve' (AUC) by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	20
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.0112 ^[10]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	101.9

Confidence interval	
level	95 %
sides	2-sided
lower limit	26.14
upper limit	177.66
Variability estimate	Standard error of the mean
Dispersion value	36.06

Notes:

[10] - Reject the null hypothesis and conclude there are significant differences in the 'area under the curve' between arms (GMSCF and Placebo) without adjustment for other covariates.

Statistical analysis title	Adjusted difference phagocyte by Area under curve
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Statistical analysis description:

Secondary outcome measure - Neutrophil capacity measured as 'area under curve' (AUC) up to day 8/9. Difference in the 'area under the curve' adjusted for the effects of site and baseline neutrophil phagocytic capacity

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	20
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.137 ^[11]
Method	ANCOVA

Notes:

[11] - Adjusted for effects of site and baseline neutrophil phagocytic capacity dependency, no significant difference in mean neutrophil phagocytic capacity as described by AUC. No site effect (p=0.4974) but evidence of baseline dependency (p=0.0323).

Secondary: Reactive oxygen species (ROS) generation –primed cells

End point title	Reactive oxygen species (ROS) generation –primed cells
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End point description:

Biological measure. Other assessments of neutrophil function: Reactive oxygen species (ROS) generation (continuous measure)

End point type	Secondary
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End point timeframe:

2 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	17		
Units: nmoles of superoxide				
arithmetic mean (standard deviation)	1.66 (± 1)	1.45 (± 1.01)		

Statistical analyses

Statistical analysis title	Unadjusted difference in ROS primed cells
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Statistical analysis description:

Secondary outcome measure - Reactive oxygen species (ROS) generation –primed cellsWe used the 2 sample t test to assess difference between the reactive oxygen species (ROS) generation (primed cells) 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	32
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.5606 ^[12]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	0.2091
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.5165
upper limit	0.9347
Variability estimate	Standard error of the mean
Dispersion value	0.3553

Notes:

[12] - Accept the null hypothesis and conclude there is no significant difference in the reactive oxygen species (ROS) generation (primed cells) between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted difference in ROS primed cells
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Statistical analysis description:

Secondary outcome measure - Reactive oxygen species (ROS) generation –primed cells
Difference in mean neutrophil phagocytic capacity at day 2 adjusted for the effects of site and baseline reactive oxygen species (ROS) generation (primed cells)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	32
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.913 ^[13]
Method	ANCOVA

Notes:

[13] - Adjusted for the effects of site and baseline (ROS) generation (primed cells), no significant difference. No site effect (p=0.6529) or Baseline dependence (p=0.8255)

Secondary: Reactive oxygen species (ROS) generation – second stimulus

End point title	Reactive oxygen species (ROS) generation – second stimulus
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End point description:

Biological measure. Other assessments of neutrophil function: Reactive oxygen species (ROS) generation (continuous measure)

End point type	Secondary
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End point timeframe:

2 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	17		
Units: nmoles of superoxide				
arithmetic mean (standard deviation)	2.49 (± 1.46)	2.05 (± 1.42)		

Statistical analyses

Statistical analysis title	Unadjusted difference ROS primed cell 2nd stimulus
Statistical analysis description: Secondary outcome measure - Reactive oxygen species (ROS) generation (second stimulus) 2 days after administration of GM-CSF/placebo. We used the 2 sample t test to assess difference between the reactive oxygen species (ROS) generation (second stimulus) 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo)	
Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	32
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.392 ^[14]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	0.4416
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.5968
upper limit	1.48
Variability estimate	Standard error of the mean
Dispersion value	0.5085

Notes:

[14] - Accept the null hypothesis and conclude there is no significant difference in the mean reactive oxygen species (ROS) generation (second stimulus) between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted difference ROS primed cells 2nd stimulus
Statistical analysis description: Secondary outcome measure - Reactive oxygen species (ROS) generation (second stimulus) 2 days after administration of GM-CSF/placebo. Difference in mean neutrophil phagocytic capacity at day 2 adjusted for the effects of site and baseline reactive oxygen species (ROS) generation (second stimulus)	
Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	32
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.733 ^[15]
Method	ANCOVA

Notes:

[15] - Adjusted for the effects of site and baseline ROS generation (2nd stimulus), no significant difference in mean ROS generation (2nd stimulus) (P=0.7330). No site effect (p=0.6269) or Baseline dependency (p=0.3343)

Secondary: Distance migrated on chemotaxis assay

End point title	Distance migrated on chemotaxis assay
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End point description:

Biological measure. Other assessments of neutrophil function: Distance migrated on chemotaxis assay

End point type	Secondary
End point timeframe:	
2 days after baseline measure and administration of treatment	

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	12	14		
Units: micrometers				
arithmetic mean (standard deviation)	415.15 ($\pm$ 230.51)	374.66 ($\pm$ 268.81)		

Statistical analyses

Statistical analysis title	Unadjusted Distance migrated on chemotaxis assay
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Statistical analysis description:

Secondary outcome measure - Distance migrated on chemotaxis assay

We used the 2 sample Mann Whitney test to assess difference between chemotaxis assay 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	26
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.5368 ^[16]
Method	Wilcoxon (Mann-Whitney)

Notes:

[16] - Accept the null hypothesis and conclude there is no significant difference in the mean distance migrated on chemotaxis assay between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted Distance migrated on chemotaxis assay
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Statistical analysis description:

Secondary outcome measure - Distance migrated on chemotaxis assay.

Difference in mean distance migrated on chemotaxis assay at day 2 adjusted for the effects of site and baseline distance migrated on chemotaxis assay

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	26
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.7945 ^[17]
Method	ANCOVA

Notes:

[17] - Adjusted for the effects of site and baseline distance migrated on chemotaxis assay, there is no significant difference in mean distance migrated on chemotaxis assay (P=0.7945). No site effect (p=0.5686) but apparent Baseline dependence (p=0.0379)

Secondary: Early apoptosis - Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria)

End point title	Early apoptosis - Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria)
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End point description:

Biological measure. Other assessments of neutrophil function: Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria)

End point type	Secondary
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End point timeframe:

2 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	17		
Units: percentage of cells				
arithmetic mean (standard deviation)	15.14 (± 9.3)	16.61 (± 8.23)		

Statistical analyses

Statistical analysis title	Unadjusted Early apoptotic rate
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Statistical analysis description:

Secondary outcome measure – Early apoptosis, Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria). We used the 2 sample t test to assess difference between early apoptotic rate 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	32
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.6395 ^[18]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	-1.4659
Confidence interval	
level	95 %
sides	2-sided
lower limit	-7.7922
upper limit	4.8604
Variability estimate	Standard error of the mean
Dispersion value	3.0977

Notes:

[18] - Accept the null hypothesis and conclude there is no significant difference in the early apoptotic rate between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted Early apoptotic rate
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Statistical analysis description:

Secondary outcome measure – Early apoptosis, Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria).

Difference in mean apoptotic rate (late apoptosis) at day 2 adjusted for the effects of site and baseline early apoptotic rate.

Comparison groups	GMCSF v Placebo
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Number of subjects included in analysis	32
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.7582 ^[19]
Method	ANCOVA

Notes:

[19] - Adjusted for the effects of site and baseline early apoptotic rate there is no significant difference in mean early apoptotic rate (P=0.7582). No site effect (p=0.6786) or Baseline dependence (p=0.2308)

Secondary: Late apoptosis - Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria)

End point title	Late apoptosis - Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria)
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End point description:

Biological measure. Other assessments of neutrophil function: Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria)

End point type	Secondary
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End point timeframe:

2 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	17		
Units: percentage of cells				
arithmetic mean (standard deviation)	7.92 (± 7.99)	10.12 (± 7.32)		

Statistical analyses

Statistical analysis title	Unadjusted late apoptotic rate
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Statistical analysis description:

Secondary outcome measure – Late apoptosis, Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria)

We used the 2 sample Mann Whitney test to assess difference between the late apoptotic rate 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo).

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	32
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.2493 ^[20]
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Mean difference (final values)
Confidence interval	
level	95 %
sides	2-sided
Variability estimate	Standard error of the mean

Notes:

[20] - Accept the null hypothesis and conclude there is no significant difference in the early apoptotic rate between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates

Statistical analysis title	Adjusted late apoptotic rate
Statistical analysis description: Secondary outcome measure – Late apoptosis, Apoptotic rate (proportion of cells identified as apoptotic by flow cytometric criteria, and/or by morphological criteria) Difference in mean apoptotic rate (late apoptosis) at day 2 adjusted for the effects of site and baseline late apoptotic rate	
Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	32
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.4053 ^[21]
Method	ANCOVA

Notes:

[21] - Adjusted for the effects of site and baseline late apoptotic rate, there is no significant difference in mean late apoptotic rate (P=0.4053). No site effect (p=0.3252) or Baseline dependence (p=0.5647)

Secondary: Sequential monocyte HLA-DR expression (QTB results)

End point title	Sequential monocyte HLA-DR expression (QTB results)
End point description: Biological measure. Other assessments of neutrophil function: Sequential monocyte HLA-DR expression (QTB results)	
End point type	Secondary
End point timeframe: 2 days after baseline measure and administration of treatment	

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	18		
Units: Antibody per cell				
arithmetic mean (standard deviation)	54998.86 (± 31239.32)	6096.51 (± 4500.89)		

Statistical analyses

Statistical analysis title	Unadjusted - Sequential monocyte HLA-DR
Statistical analysis description: Secondary outcome measure - Sequential monocyte HLA-DR expression (QTB results) We used the 2 sample Mann Whitney test to assess difference between the APC (antibody bound per cell) 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo).	
Comparison groups	Placebo v GMCSF

Number of subjects included in analysis	31
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.0012 ^[22]
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Mean difference (final values)
Confidence interval	
sides	2-sided

Notes:

[22] - Reject the null hypothesis and conclude there are significant difference in the mean APC between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted - Sequential monocyte HLA-DR
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Statistical analysis description:

Secondary outcome measure - Sequential monocyte HLA-DR expression (QTB results)

Difference in mean between the APC (antibody bound per cell) 2 days after administration of GM-CSF/placebo by arm at day 2 adjusted for the effects of site and baseline APC.

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	31
Analysis specification	Pre-specified
Analysis type	superiority
P-value	< 0 ^[23]
Method	ANCOVA

Notes:

[23] - Adjusted for the effects of site and baseline antibody bound per cell (APC), there are significant differences in mean APC (P=0.0000). No site effect (p=0.4692) but evidence of baseline dependence (p=0.0100).

Secondary: CD88 - (relative median fluorescence)

End point title	CD88 - (relative median fluorescence)
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End point description:

Biological measure. Other assessments of neutrophil function: relative median fluorescence (CD88 results)

End point type	Secondary
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End point timeframe:

2 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	12	18		
Units: ratio				
arithmetic mean (standard deviation)	4.24 (± 1.45)	4.8 (± 2.56)		

Statistical analyses

Statistical analysis title	Uadjusted relative median fluorescence 2 days
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Statistical analysis description:

Secondary outcome measure - CD88 (relative median fluorescence) 2 days after administration of GM-

CSF/placebo.

We used the 2 sample t test to assess difference between the CD88 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	30
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.4997 [24]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	-0.5581
Confidence interval	
level	95 %
sides	2-sided
lower limit	-2.2299
upper limit	1.1137
Variability estimate	Standard error of the mean
Dispersion value	0.8162

Notes:

[24] - Accept the null hypothesis and conclude there is no significant difference in the mean CD88 between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	adjusted relative median fluorescence
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Statistical analysis description:

Secondary outcome measure - CD88 (relative median fluorescence) 2 days after administration of GM-CSF/placebo.

Difference in mean CD88 at day 2 adjusted for the effects of site and baseline CD88 measures

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	30
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.3924 [25]
Method	ANCOVA

Notes:

[25] - Adjusted for the effects of site and baseline CD88 expression, there is no significant difference in mean CD88 (P=0.3924). No site effect (p=0.8062) or Baseline dependence (p=0.0595)

Secondary: Percentage of T cells (naïve/memory t reg cells) CD45RA+RO-

End point title	Percentage of T cells (naïve/memory t reg cells) CD45RA+RO-
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End point description:

Biological measure. Other assessments of neutrophil function: Percentage of T cells (naïve/memory t reg cells) CD45RA+RO-

End point type	Secondary
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End point timeframe:

2 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	12	15		
Units: percentage				
arithmetic mean (standard deviation)	35.63 ($\pm$ 12.24)	34.66 ($\pm$ 14.48)		

Statistical analyses

Statistical analysis title	Unadjusted CD45RA+RO-
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Statistical analysis description:

Secondary outcome measure - Percentage of T cells (naïve/memory t reg cells) CD45RA+RO-
We used the 2 sample t test to assess difference between the CD45RA+RO- 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	27
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.8555 ^[26]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	0.965
Confidence interval	
level	95 %
sides	2-sided
lower limit	-9.835
upper limit	11.765
Variability estimate	Standard error of the mean
Dispersion value	5.244

Notes:

[26] - Accept the null hypothesis and conclude there is no significant difference in the mean CD45RA+RO- between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted CD45RA+RO-
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Statistical analysis description:

Secondary outcome measure - Percentage of T cells (naïve/memory t reg cells) CD45RA+RO-.
Difference in mean CD45RA+RO-.at day 2 adjusted for the effects of site and baseline CD45RA+RO-.

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	27
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.763 ^[27]
Method	ANCOVA

Notes:

[27] - Adjusted for the effects of site and baseline CD45RA+RO-, there is no significant difference in mean CD45RA+RO- (P=0.7630). No site effect (p=0.1729) but evidence of baseline dependence (p=0.0000).

Secondary: Percentage of T cells (naïve/memory t reg cells) CD45RO+RA-

End point title	Percentage of T cells (naïve/memory t reg cells) CD45RO+RA-
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End point description:

Biological measure. Other assessments of neutrophil function: Percentage of T cells (naïve/memory t reg cells)

CD45RO+RA-

End point type	Secondary
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End point timeframe:

2 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	12	15		
Units: percent				
arithmetic mean (standard deviation)	49.57 (± 13.89)	48.25 (± 17.53)		

Statistical analyses

Statistical analysis title	Unadjusted CD45RO+RA-
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Statistical analysis description:

Secondary outcome measure - Percentage of T cells (naïve/memory t reg cells) CD45RO+RA-

We used the 2 sample t test to assess difference between the CD45RO+RA- 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
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Number of subjects included in analysis	27
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Analysis specification	Pre-specified
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Analysis type	superiority
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P-value	= 0.8334 [28]
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Method	t-test, 2-sided
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Parameter estimate	Mean difference (final values)
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Point estimate	1.32
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Confidence interval

level	95 %
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sides	2-sided
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lower limit	-11.467
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upper limit	14.107
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Variability estimate	Standard error of the mean
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Dispersion value	6.209
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Notes:

[28] - Accept the null hypothesis and conclude there is no significant difference in the mean CD45RO+RA- between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted CD45RO+RA-
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Statistical analysis description:

Secondary outcome measure - Percentage of T cells (naïve/memory t reg cells) CD45RO+RA-

Difference in mean CD45RO+RA- at day 2 adjusted for the effects of site and baseline CD45RO+RA-

Comparison groups	Placebo v GMCSF
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Number of subjects included in analysis	27
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.5344 ^[29]
Method	ANCOVA

Notes:

[29] - Adjusted for the effects of site and baseline CD45RO+RA-, there is no significant difference in mean CD45RO+RA- (P=0.5344). No site effect (p=0.1966) but evidence of baseline dependence (p=0.0001).

Secondary: Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-6

End point title	Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-6
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End point description:

Biological measure. Other assessments of neutrophil function: Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-6

End point type	Secondary
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End point timeframe:

2 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	19		
Units: pg/ml				
arithmetic mean (standard deviation)	94.16 (± 115.85)	504.57 (± 1400.2)		

Statistical analyses

Statistical analysis title	Unadjusted IL-6
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Statistical analysis description:

Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-6

We used the 2 sample Mann Whitney test to assess difference between IL-6 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo).

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.8842 ^[30]
Method	Wilcoxon (Mann-Whitney)

Notes:

[30] - Accept the null hypothesis and conclude there is no significant difference in the mean IL-6 between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted IL-6
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Statistical analysis description:

Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-6

Difference in mean IL-6 at day 2 adjusted for the effects of site and baseline IL-6

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.4031 ^[31]
Method	ANCOVA

Notes:

[31] - Adjusted for the effects of site and baseline IL-6, there is no significant difference in mean IL-6 (P=0.4031). No site effect (p=0.5312) but evidence of baseline dependence (p=0.0000).

Secondary: Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-8

End point title	Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-8
End point description:	Biological measure. Other assessments of neutrophil function: Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-8
End point type	Secondary
End point timeframe:	2 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	19		
Units: pg/ml				
arithmetic mean (standard deviation)	131.73 (± 155.18)	314.66 (± 546.91)		

Statistical analyses

Statistical analysis title	Ajusted IL-8
Statistical analysis description:	Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-8 Difference in mean IL-8 at day 2 adjusted for the effects of site and baseline IL-8
Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.6575 ^[32]
Method	ANCOVA

Notes:

[32] - Adjusted for the effects of site and baseline IL-8, there is no significant difference in mean IL-8 (P=0.6575). No site effect (p=0.2552) but evidence of baseline dependence (p=0.0000).

Statistical analysis title	Unadjusted IL-8
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Statistical analysis description:

Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum -

IL-8.

We used the 2 sample Mann Whitney test to assess difference between IL-8 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo).

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.1897 ^[33]
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[33] - Accept the null hypothesis and conclude there is no significant difference in the mean IL-8 between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Secondary: Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-1 β

End point title	Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-1 β
End point description: Biological measure. Other assessments of neutrophil function: Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-1 β	
End point type	Secondary
End point timeframe: 2 days after baseline measure and administration of treatment	

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	19		
Units: pg/ml				
arithmetic mean (standard deviation)	8.38 ($\pm$ 14.71)	10.31 ($\pm$ 15.73)		

Statistical analyses

Statistical analysis title	Unadjusted IL-1 β
Statistical analysis description: Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-1 β . We used the 2 sample Mann Whitney test to assess difference between IL-1 β 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo).	
Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.0654 ^[34]
Method	Wilcoxon (Mann-Whitney)

Notes:

[34] - Accept the null hypothesis and conclude there is no significant difference in the mean IL-1 β between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted IL-1 β
Statistical analysis description:	
Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-1 β .	
Difference in mean IL-1 β at day 2 adjusted for the effects of site and baseline IL-1 β	
Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.3268 ^[35]
Method	ANCOVA

Notes:

[35] - Adjusted for the effects of site and baseline IL-1 β there is no significant difference in mean IL-1 β (P=0.3268). No site effect (p=0.8772) or evidence of baseline dependence (p=0.1327).

Secondary: Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-10

End point title	Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-10
End point description:	
Biological measure. Other assessments of neutrophil function: Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-10	
End point type	Secondary
End point timeframe:	
2 days after baseline measure and administration of treatment	

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	19		
Units: pg/ml				
arithmetic mean (standard deviation)	14.95 ($\pm$ 21.1)	9.64 ($\pm$ 13.79)		

Statistical analyses

Statistical analysis title	Unadjusted IL-10
Statistical analysis description:	
Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-10.	
We used the 2 sample Mann Whitney test to assess difference between IL-10 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo).	
Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.2002 ^[36]
Method	Wilcoxon (Mann-Whitney)

Notes:

[36] - Accept the null hypothesis and conclude there is no significant difference in the mean IL-10 between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted IL-10
Statistical analysis description: Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-10. Difference in mean IL-10 at day 2 adjusted for the effects of site and baseline IL-10	
Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.2583 [37]
Method	ANCOVA

Notes:

[37] - Adjusted for the effects of site and baseline IL-10 there is no significant difference in mean IL-10 (P=0.2583). No site effect (p=0.4597) but evidence of baseline dependence (p=0.0000).

Secondary: Cytokine data measure of pro/anti-inflammatory mediators in serum - TNF

End point title	Cytokine data measure of pro/anti-inflammatory mediators in serum - TNF
End point description: Biological measure. Other assessments of neutrophil function: Cytokine data measure of pro/anti-inflammatory mediators in serum - TNF	
End point type	Secondary
End point timeframe: 2 days after baseline measure and administration of treatment	

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	19		
Units: pg/ml				
arithmetic mean (standard deviation)	9.06 (± 13.28)	4.3 (± 9.52)		

Statistical analyses

Statistical analysis title	Unadjusted TNF
Statistical analysis description: Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - TNF. We used the 2 sample Mann Whitney test to assess difference between TNF 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo).	
Comparison groups	GMCSF v Placebo

Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.1687 ^[38]
Method	Wilcoxon (Mann-Whitney)

Notes:

[38] - Accept the null hypothesis and conclude there is no significant difference in the mean TNF between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted TNF
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Statistical analysis description:

Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - TNF

Difference in mean TNF at day 2 adjusted for the effects of site and baseline TNF

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.2241 ^[39]
Method	ANCOVA

Notes:

[39] - Adjusted for the effects of site and baseline TNF there is no significant difference in mean TNF (P=0.2241). No site effect (p=0.4524) but evidence of baseline dependence (p=0.0023).

Secondary: Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-12 p70

End point title	Cytokine data measure of pro/anti-inflammatory mediators in serum -IL-12 p70
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End point description:

Biological measure. Other assessments of neutrophil function: Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-12 p70

End point type	Secondary
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End point timeframe:

2 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	19		
Units: pg/ml				
arithmetic mean (standard deviation)	1.92 (± 4.04)	1.27 (± 2.9)		

Statistical analyses

Statistical analysis title	Unadjusted IL-12 p70
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Statistical analysis description:

Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-12 p70.

We used the 2 sample Mann Whitney test to assess difference between IL-12 p70 2 days after administration of GM-CSF/placebo by arm (GMCSF v Placebo).

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.4662 ^[40]
Method	Wilcoxon (Mann-Whitney)

Notes:

[40] - Accept the null hypothesis and conclude there is no significant difference in the mean IL-12 p70 between arms 2 days after administration of GM-CSF/placebo without adjustment for other covariates.

Statistical analysis title	Adjusted IL-12 p70
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Statistical analysis description:

Secondary outcome measure - Cytokine data measure of pro/anti-inflammatory mediators in serum - IL-12 p70.

Difference in mean IL-12 p70 at day 2 adjusted for the effects of site and baseline IL-12 p70

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.6186 ^[41]
Method	ANCOVA

Notes:

[41] - Adjusted for the effects of site and baseline IL-12 p70 there is no significant difference in mean IL-12 p70 (P=0.6186). No site effect (p=0.9487) but evidence of baseline dependence (p=0.0000).

Secondary: sequential organ failure assessment (SOFA) at day 2

End point title	sequential organ failure assessment (SOFA) at day 2
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End point description:

sequential organ failure assessment (SOFA) at day 2

End point type	Secondary
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End point timeframe:

2 days after treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	15	21		
Units: integer				
median (inter-quartile range (Q1-Q3))	7 (2 to 11)	7 (5 to 9)		

Statistical analyses

No statistical analyses for this end point

Secondary: incidence of ICU acquired infections

End point title	incidence of ICU acquired infections
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End point description:

Hospitals in Europe link for infection control through surveillance.

incidence of ICU acquired infection (ICUIAs)i

End point type	Secondary
End point timeframe:	
2 days after administration of treatment	

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	17	21		
Units: count				
number (not applicable)	0	0		

Statistical analyses

No statistical analyses for this end point

Secondary: All cause mortality 30 days post randomisation

End point title	All cause mortality 30 days post randomisation
End point description:	
All cause mortality 30 days post randomisation	
End point type	Secondary
End point timeframe:	
30 days after randomization to the trial	

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	17	21		
Units: number	4	6		

Statistical analyses

No statistical analyses for this end point

Post-hoc: Neutrophil capacity measured as 'area under curve' (AUC) up to day4/5

End point title	Neutrophil capacity measured as 'area under curve' (AUC) up to day4/5
End point description:	
Area under curve is calculated by summing the areas between each time point. This procedure is known as the linear trapezoidal rule.	
In cases where there is missing data with no more data either before or after that point we do not calculate the area and treat that patient's area under curve as missing.	
The area under the curve up to day 4/5 is calculated for each patient. The summary statistics split by arm are then calculated.	

End point type	Post-hoc
End point timeframe: up to day4/5 after administration of treatment	

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	12	16		
Units: cells x 109/l x days				
arithmetic mean (standard deviation)	280.3 (± 56.1)	234.9 (± 43.2)		

Statistical analyses

Statistical analysis title	Unadjusted diff phagocyte Area under curve 4/5day
Statistical analysis description:	
Ad hoc outcome measure - Neutrophil capacity measured as 'area under curve' (AUC) up to day 4/5.	
Secondary outcome measure - Neutrophil capacity measured as 'area under curve' (AUC) up to day 4/5.	
Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	28
Analysis specification	Post-hoc
Analysis type	superiority
P-value	= 0.0227 ^[42]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	45.4
Confidence interval	
level	95 %
sides	2-sided
lower limit	6.86
upper limit	83.94
Variability estimate	Standard error of the mean
Dispersion value	18.75

Notes:

[42] - Reject the null hypothesis and conclude there are significant differences in the 'area under the curve' up to day 4/5 between arms (GMCSF and Placebo) without adjustment for other covariates.

Statistical analysis title	Adjusted diffs phagocyte Area under curve 4/5days
Statistical analysis description:	
Ad hoc outcome measure - Neutrophil capacity measured as 'area under curve' (AUC) up to day 4/5.	
Difference in the 'area under the curve' adjusted for the effects of site and baseline neutrophil phagocytic capacity	
Comparison groups	GMCSF v Placebo

Number of subjects included in analysis	28
Analysis specification	Post-hoc
Analysis type	superiority
P-value	= 0.2813 ^[43]
Method	ANCOVA

Notes:

[43] - Adjusted for effects of site and baseline neutrophil phagocytic capacity, no significant difference in mean neutrophil phagocytic capacity as described by AUC up to day 4/5. There is site effect (p=0.0216) and evidence baseline dependency (p=0.0001)

Post-hoc: Area under curve for Leukocytes (WCC) up to day 8/9

End point title	Area under curve for Leukocytes (WCC) up to day 8/9
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End point description:

Area under curve is calculated by summing the areas between each time point. This procedure is known as the linear trapezoidal rule.

In cases where there is missing data with no more data either before or after that point we do not calculate the area and treat that patient's area under curve as missing.

The area under the curve up to day 8/9 is calculated for each patient. The summary statistics split by arm are then calculated

End point type	Post-hoc
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End point timeframe:

8/9 days after baseline measure and administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	10	15		
Units: cells x 10 ⁹ /l x days				
arithmetic mean (standard deviation)	160.2 (± 35.3)	111.9 (± 34.5)		

Statistical analyses

Statistical analysis title	Unadjusted diff Leukocytes Area under curve 8/9day
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Statistical analysis description:

Secondary outcome measure - Area under curve for Leukocytes (WCC) up to day 8/9.

We used the 2 sample t test to assess difference between the 'area under the curve' for Leukocytes (LAUC) for day 0 to day 9 for all patients by arm (GMCSF v Placebo)

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	25
Analysis specification	Post-hoc
Analysis type	superiority
P-value	= 0.0025 ^[44]
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)
Point estimate	48.25

Confidence interval	
level	95 %
sides	2-sided
lower limit	18.87
upper limit	77.63
Variability estimate	Standard error of the mean
Dispersion value	14.2

Notes:

[44] - Reject the null hypothesis and conclude there are significant differences in the 'area under the curve' for leukocytes between arms (GMCSF and Placebo) without adjustment for other covariates.

Statistical analysis title	Adjusted diffs Leukocytes Area under curve 8/9day
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Statistical analysis description:

Ad hoc outcome measure - Area under curve for Leukocytes (WCC) up to day 8/9

Difference in the 'area under the curve' adjusted for the effects of site and baseline leukocyte dependency

Comparison groups	GMCSF v Placebo
Number of subjects included in analysis	25
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.0039 [45]
Method	ANCOVA

Notes:

[45] - Adjusted for the effects of site and baseline leukocytes, significant differences in mean leukocytes as described by area under curve day0 to 9 (P=0.0039). No site effect (p=0.0722) but there is evidence of baseline leukocyte dependency (p=0.0427)

Post-hoc: ratio of lowest PaO2 to FiO2 at day 2

End point title	ratio of lowest PaO2 to FiO2 at day 2
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End point description:

ratio of lowest PaO2 to FiO2 at day 2

End point type	Post-hoc
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End point timeframe:

2 days after administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	14	20		
Units: ratio				
arithmetic mean (standard deviation)	27.6 (± 9.7)	27.3 (± 12.7)		

Statistical analyses

No statistical analyses for this end point

Post-hoc: ratio of highest PaO2 to FiO2 at day 2

End point title	ratio of highest PaO2 to FiO2 at day 2
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End point description:

ratio of highest PaO₂ to FiO₂ at day 2

End point type

Post-hoc

End point timeframe:

2 days after administration of treatment

End point values	GMCSF	Placebo		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	14	20		
Units: ratio				
arithmetic mean (standard deviation)	37 (± 15.3)	38.8 (± 15.6)		

Statistical analyses

No statistical analyses for this end point

Adverse events

Adverse events information

Timeframe for reporting adverse events:

Adverse events were reported from first visit until final visit

Adverse event reporting additional description:

Researchers reviewed and recorded any adverse events on a daily basis from Day 0 to Day 9.

Assessment type	Systematic
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Dictionary used

Dictionary name	as reported verbatim
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Dictionary version	0
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Reporting groups

Reporting group title	Leukine
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Reporting group description:

Participants randomised to GMCSF arm study will receive 4 days of treatment with 250 microgram/vial) Leukine as a subcutaneous injection. Dosed on actual body weight up to a maximum dose of 450microgram.

Reporting group title	Placebo
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Reporting group description:

Participants randomised to placebo arm study will receive 4 days of treatment with 0.9% sodium chloride (placebo, 5ml /ampoule) as a subcutaneous injection. Dosed on actual body weight up to a maximum dose of volume 1.8ml.

Serious adverse events	Leukine	Placebo	
Total subjects affected by serious adverse events			
subjects affected / exposed	1 / 17 (5.88%)	2 / 21 (9.52%)	
number of deaths (all causes)	4	6	
number of deaths resulting from adverse events	0	0	
Surgical and medical procedures			
desaturation	Additional description: sudden desaturation while ventilated. Required 100% o2 and endotracheal tube change		
subjects affected / exposed	0 / 17 (0.00%)	1 / 21 (4.76%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Gastrointestinal disorders			
Death	Additional description: admitted with bowel obstruction. Initially managed conservatively. underwent hemicolectomy. continued deterioration and Rx withdrawn		
subjects affected / exposed	1 / 17 (5.88%)	0 / 21 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 1	0 / 0	
Respiratory, thoracic and mediastinal disorders			
Hypoxia	Additional description: worsening acute hypoxic / hypercapnic respiratory failure		

subjects affected / exposed	0 / 17 (0.00%)	1 / 21 (4.76%)	
occurrences causally related to treatment / all	0 / 0	1 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	

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

Non-serious adverse events	Leukine	Placebo	
Total subjects affected by non-serious adverse events			
subjects affected / exposed	11 / 17 (64.71%)	4 / 21 (19.05%)	
Investigations			
Blood alkaline phosphatase increased			
subjects affected / exposed	1 / 17 (5.88%)	0 / 21 (0.00%)	
occurrences (all)	1	0	
Transaminases abnormal	Additional description: increase in hepatic transaminases ALT and AST		
subjects affected / exposed	3 / 17 (17.65%)	0 / 21 (0.00%)	
occurrences (all)	3	0	
Pyrexia	Additional description: Pyrexia up 39.1 day 2, 39.5 day 3 and 39.8 day 3, same patient		
subjects affected / exposed	1 / 17 (5.88%)	0 / 21 (0.00%)	
occurrences (all)	3	0	
Blood and lymphatic system disorders			
Thrombocytopenia			
subjects affected / exposed	2 / 17 (11.76%)	1 / 21 (4.76%)	
occurrences (all)	2	1	
General disorders and administration site conditions			
internal jugular vein thrombus			
subjects affected / exposed	1 / 17 (5.88%)	1 / 21 (4.76%)	
occurrences (all)	1	1	
fever	Additional description: Fever up to 38 degrees. Resolved with paracetamol		
subjects affected / exposed	1 / 17 (5.88%)	0 / 21 (0.00%)	
occurrences (all)	1	0	
transient hypoglycaemia			
subjects affected / exposed	0 / 17 (0.00%)	1 / 21 (4.76%)	
occurrences (all)	0	1	
increased oxygen	Additional description: Increased oxygen requirements on background of presumed necrotizing pneumonia with in situ thrombus.		

subjects affected / exposed	0 / 17 (0.00%)	1 / 21 (4.76%)	
occurrences (all)	0	1	

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
18 October 2013	conditions in place to allow IMP to be made up on site and delivered to blinded study team members

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.

-number of patients screened relative to the number recruited -assay used labour-intensive and operator-dependent
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Notes: