



Clinical trial results:

A randomized, placebo-controlled, double-blind study to evaluate safety and dose dependent clinical efficacy of APO-2 at three different doses in patients with diabetic foot ulcer (MARSYAS II)

Summary

EudraCT number	2018-001653-27
Trial protocol	AT CZ DE
Global end of trial date	06 December 2023

Results information

Result version number	v1 (current)
This version publication date	28 February 2025
First version publication date	28 February 2025

Trial information

Trial identification

Sponsor protocol code	Marsyas II
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Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	NCT04277598
WHO universal trial number (UTN)	-

Notes:

Sponsors

Sponsor organisation name	Aposcience AG
Sponsor organisation address	Dresdner Strasse 87/A 21, Wien, Austria, 1200
Public contact	Aposcience AG, Aposcience AG, +43 (0)664 212 05 57, info@aposcience.com
Scientific contact	Aposcience AG, Aposcience AG, +43 (0)664 212 05 57, info@aposcience.com

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 December 2023
Is this the analysis of the primary completion data?	Yes
Primary completion date	06 December 2023
Global end of trial reached?	Yes
Global end of trial date	06 December 2023
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

To determine the dose-response for clinical efficacy of APO-2 multiple dose administration in patients with diabetic foot ulcer at three different dose levels compared to placebo.

Protection of trial subjects:

The study was conducted in full conformance with the principles of the Declaration of Helsinki (as amended at the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013) and with the laws and regulations of the country in which the clinical research was conducted. Only appropriately trained personnel were involved in the study. The study followed the International Council for Harmonisation Good Clinical Practice Guideline and the European Directive embedded in the Austrian, German, Czech and Polish drug act. Before any clinical study-related activities were performed, the investigator reviewed the informed consent form and explained the study to the patient. The investigator ensured that the patient was fully informed about the nature, significance, impact, and risks of the study.

Background therapy:

Standard of care

Evidence for comparator: -

Actual start date of recruitment	11 November 2020
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	Poland: 53
Country: Number of subjects enrolled	Austria: 22
Country: Number of subjects enrolled	Czechia: 46
Country: Number of subjects enrolled	Germany: 1
Worldwide total number of subjects	122
EEA total number of subjects	122

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)	67
From 65 to 84 years	55
85 years and over	0

Subject disposition

Recruitment

Recruitment details:

Patients were recruited between 11-Nov-2020 and 4-Sep-2023.

Pre-assignment

Screening details:

The study started with a safety lead-in phase during which 15 patients were screened. In the subsequent main phase, 144 patients were screened. Of the 159 screened patients, 122 patients were treated with APO-2 or placebo.

Period 1

Period 1 title	Overall trial (overall period)
Is this the baseline period?	Yes
Allocation method	Randomised - controlled
Blinding used	Double blind
Roles blinded	Subject, Investigator, Monitor, Assessor

Blinding implementation details:

Site personnel, including the investigator and site personnel administering the investigational medicinal product (IMP), central assessors of wound area, and the patients were blinded to the treatment allocation. Unblinded site personnel (pharmacists) prepared the IMP for administration.

Arms

Are arms mutually exclusive?	No
Arm title	APO-2 12.5 U/mL (FAS)

Arm description:

Patients in the full analysis set (FAS) who were administered APO-2 12.5 U/mL hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Arm type	Experimental
Investigational medicinal product name	APO-2
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Gel
Routes of administration	Topical

Dosage and administration details:

APO-2 was supplied as a frozen concentrate for cutaneous solution containing 100 U APOSEC(TM) dissolved in 0.5 mL of 0.9% saline solution. APOSEC(TM) is the secretome of stressed peripheral blood mononuclear cells in CellGenix Good Manufacturing Practice Dendritic Cell medium (matrix). APO-2 was diluted with 0.9% saline solution and mixed with NU-GEL up to a final volume of 4 mL at a dose of 12.5 U/mL. The reconstituted IMP was applied 3 times per week (within 7 days, at least 1 day apart) for 4 weeks topically on the wound surface area using a syringe and a spatula as an applicator. The amount of IMP applied depended on the wound area and ranged from 0.2 to 4.0 mL.

Arm title	APO-2 25.0 U/mL (FAS)
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Arm description:

Patients in the FAS who were administered APO-2 25.0 U/mL hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Arm type	Experimental
Investigational medicinal product name	APO-2
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Gel
Routes of administration	Topical

Dosage and administration details:

APO-2 was supplied as a frozen concentrate for cutaneous solution containing 100 U APOSEC(TM) dissolved in 0.5 mL of 0.9% saline solution. APOSEC(TM) is the secretome of stressed peripheral blood mononuclear cells in CellGenix Good Manufacturing Practice Dendritic Cell medium (matrix). APO-2 was diluted with 0.9% saline solution and mixed with NU-GEL up to a final volume of 4 mL at a dose of 25.0 U/mL. The reconstituted IMP was applied 3 times per week (within 7 days, at least 1 day apart) for 4 weeks topically on the wound surface area using a syringe and a spatula as an applicator. The amount of IMP applied depended on the wound area and ranged from 0.2 to 4.0 mL.

Arm title	APO-2 50.0 U/mL (FAS)
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Arm description:

Patients in the FAS who were administered APO-2 50.0 U/mL hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Arm type	Experimental
Investigational medicinal product name	APO-2
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Gel
Routes of administration	Topical

Dosage and administration details:

APO-2 was supplied as a frozen concentrate for cutaneous solution containing 100 U APOSEC(TM) dissolved in 0.5 mL of 0.9% saline solution. APOSEC(TM) is the secretome of stressed peripheral blood mononuclear cells in CellGenix Good Manufacturing Practice Dendritic Cell medium (matrix). APO-2 was diluted with 0.9% saline solution and mixed with NU-GEL up to a final volume of 4 mL at a dose of 50.0 U/mL. The reconstituted IMP was applied 3 times per week (within 7 days, at least 1 day apart) for 4 weeks topically on the wound surface area using a syringe and a spatula as an applicator. The amount of IMP applied depended on the wound area and ranged from 0.2 to 4.0 mL.

Arm title	Placebo (FAS)
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Arm description:

Patients in the FAS who were administered the placebo hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Arm type	Placebo
Investigational medicinal product name	Placebo
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Gel
Routes of administration	Topical

Dosage and administration details:

The placebo was the processed CellGenix Good Manufacturing Practice Dendritic Cell medium (APOSEC[™] matrix) diluted with 0.9% saline solution and mixed with NU-GEL up to a final volume of 4 mL. The reconstituted IMP was applied 3 times per week (within 7 days, at least 1 day apart) for 4 weeks topically on the wound surface area using a syringe and a spatula as an applicator. The amount of IMP applied depended on the wound area and ranged from 0.2 to 4.0 mL.

Arm title	APO-2 12.5 U/mL (FAS posthoc subgroup)
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Arm description:

Patients in the FAS posthoc subgroup who were administered APO-2 12.5 U/mL hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Arm type	Experimental
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Investigational medicinal product name	APO-2
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Gel
Routes of administration	Topical

Dosage and administration details:

APO-2 was supplied as a frozen concentrate for cutaneous solution containing 100 U APOSEC(TM) dissolved in 0.5 mL of 0.9% saline solution. APOSEC(TM) is the secretome of stressed peripheral blood mononuclear cells in CellGenix Good Manufacturing Practice Dendritic Cell medium (matrix). APO-2 was diluted with 0.9% saline solution and mixed with NU-GEL up to a final volume of 4 mL at a dose of 12.5 U/mL. The reconstituted IMP was applied 3 times per week (within 7 days, at least 1 day apart) for 4 weeks topically on the wound surface area using a syringe and a spatula as an applicator. The amount of IMP applied depended on the wound area and ranged from 0.2 to 4.0 mL.

Arm title	APO-2 25.0 U/mL (FAS posthoc subgroup)
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Arm description:

Patients in the FAS posthoc subgroup who were administered APO-2 25.0 U/mL hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Arm type	Experimental
Investigational medicinal product name	APO-2
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Other name	
Pharmaceutical forms	Gel
Routes of administration	Topical

Dosage and administration details:

APO-2 was supplied as a frozen concentrate for cutaneous solution containing 100 U APOSEC(TM) dissolved in 0.5 mL of 0.9% saline solution. APOSEC(TM) is the secretome of stressed peripheral blood mononuclear cells in CellGenix Good Manufacturing Practice Dendritic Cell medium (matrix). APO-2 was diluted with 0.9% saline solution and mixed with NU-GEL up to a final volume of 4 mL at a dose of 25.0 U/mL. The reconstituted IMP was applied 3 times per week (within 7 days, at least 1 day apart) for 4 weeks topically on the wound surface area using a syringe and a spatula as an applicator. The amount of IMP applied depended on the wound area and ranged from 0.2 to 4.0 mL.

Arm title	APO-2 50.0 U/mL (FAS posthoc subgroup)
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Arm description:

Patients in the FAS posthoc subgroup who were administered APO-2 50.0 U/mL hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Arm type	Experimental
Investigational medicinal product name	APO-2
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Gel
Routes of administration	Topical

Dosage and administration details:

APO-2 was supplied as a frozen concentrate for cutaneous solution containing 100 U APOSEC(TM) dissolved in 0.5 mL of 0.9% saline solution. APOSEC(TM) is the secretome of stressed peripheral blood mononuclear cells in CellGenix Good Manufacturing Practice Dendritic Cell medium (matrix). APO-2 was diluted with 0.9% saline solution and mixed with NU-GEL up to a final volume of 4 mL at a dose of 50.0

U/mL. The reconstituted IMP was applied 3 times per week (within 7 days, at least 1 day apart) for 4 weeks topically on the wound surface area using a syringe and a spatula as an applicator. The amount of IMP applied depended on the wound area and ranged from 0.2 to 4.0 mL.

Arm title	Placebo (FAS posthoc subgroup)
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Arm description:

Patients in the FAS posthoc subgroup who were administered the placebo hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Arm type	Placebo
Investigational medicinal product name	Placebo
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Gel
Routes of administration	Topical

Dosage and administration details:

The placebo was the processed CellGenix Good Manufacturing Practice Dendritic Cell medium (APOSEC[™] matrix) diluted with 0.9% saline solution and mixed with NU-GEL up to a final volume of 4 mL. The reconstituted IMP was applied 3 times per week (within 7 days, at least 1 day apart) for 4 weeks topically on the wound surface area using a syringe and a spatula as an applicator. The amount of IMP applied depended on the wound area and ranged from 0.2 to 4.0 mL.

Number of subjects in period 1	APO-2 12.5 U/mL (FAS)	APO-2 25.0 U/mL (FAS)	APO-2 50.0 U/mL (FAS)
Started	27	38	26
Completed	26	37	26
Not completed	1	1	0
Adverse event, non-fatal	1	1	-

Number of subjects in period 1	Placebo (FAS)	APO-2 12.5 U/mL (FAS posthoc subgroup)	APO-2 25.0 U/mL (FAS posthoc subgroup)
	Started	31	23
Completed	30	22	26
Not completed	1	1	1
Adverse event, non-fatal	1	1	1

Number of subjects in period 1	APO-2 50.0 U/mL (FAS posthoc subgroup)	Placebo (FAS posthoc subgroup)
	Started	21
Completed	21	22
Not completed	0	1
Adverse event, non-fatal	-	1

Baseline characteristics

Reporting groups

Reporting group title	APO-2 12.5 U/mL (FAS)
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Reporting group description:

Patients in the full analysis set (FAS) who were administered APO-2 12.5 U/mL hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Reporting group title	APO-2 25.0 U/mL (FAS)
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Reporting group description:

Patients in the FAS who were administered APO-2 25.0 U/mL hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Reporting group title	APO-2 50.0 U/mL (FAS)
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Reporting group description:

Patients in the FAS who were administered APO-2 50.0 U/mL hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Reporting group title	Placebo (FAS)
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Reporting group description:

Patients in the FAS who were administered the placebo hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Reporting group title	APO-2 12.5 U/mL (FAS posthoc subgroup)
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Reporting group description:

Patients in the FAS posthoc subgroup who were administered APO-2 12.5 U/mL hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Reporting group title	APO-2 25.0 U/mL (FAS posthoc subgroup)
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Reporting group description:

Patients in the FAS posthoc subgroup who were administered APO-2 25.0 U/mL hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Reporting group title	APO-2 50.0 U/mL (FAS posthoc subgroup)
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Reporting group description:

Patients in the FAS posthoc subgroup who were administered APO-2 50.0 U/mL hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Reporting group title	Placebo (FAS posthoc subgroup)
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Reporting group description:

Patients in the FAS posthoc subgroup who were administered the placebo hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients

were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Reporting group values	APO-2 12.5 U/mL (FAS)	APO-2 25.0 U/mL (FAS)	APO-2 50.0 U/mL (FAS)
Number of subjects	27	38	26
Age categorical Units: Subjects			
In utero Preterm newborn infants (gestational age < 37 wks) Newborns (0-27 days) Infants and toddlers (28 days-23 months) Children (2-11 years) Adolescents (12-17 years) Adults (18-64 years) From 65-84 years 85 years and over			
Age continuous Units: years arithmetic mean standard deviation	59.1 ± 10.8	62.6 ± 10.4	62.5 ± 8.4
Gender categorical Units: Subjects			
Female	3	10	3
Male	24	28	23

Reporting group values	Placebo (FAS)	APO-2 12.5 U/mL (FAS posthoc subgroup)	APO-2 25.0 U/mL (FAS posthoc subgroup)
Number of subjects	31	23	27
Age categorical Units: Subjects			
In utero Preterm newborn infants (gestational age < 37 wks) Newborns (0-27 days) Infants and toddlers (28 days-23 months) Children (2-11 years) Adolescents (12-17 years) Adults (18-64 years) From 65-84 years 85 years and over			
Age continuous Units: years arithmetic mean standard deviation	62.7 ± 11.3	59.0 ± 11.4	63.4 ± 10.2

Gender categorical Units: Subjects			
Female	2	2	9
Male	29	21	18

Reporting group values	APO-2 50.0 U/mL (FAS posthoc subgroup)	Placebo (FAS posthoc subgroup)	Total
Number of subjects	21	23	122
Age categorical Units: Subjects			
In utero Preterm newborn infants (gestational age < 37 wks) Newborns (0-27 days) Infants and toddlers (28 days-23 months) Children (2-11 years) Adolescents (12-17 years) Adults (18-64 years) From 65-84 years 85 years and over			
Age continuous Units: years			
arithmetic mean	63.0	61.5	
standard deviation	± 9.0	± 11.4	-
Gender categorical Units: Subjects			
Female	2	2	18
Male	19	21	104

End points

End points reporting groups

Reporting group title	APO-2 12.5 U/mL (FAS)
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Reporting group description:

Patients in the full analysis set (FAS) who were administered APO-2 12.5 U/mL hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Reporting group title	APO-2 25.0 U/mL (FAS)
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Reporting group description:

Patients in the FAS who were administered APO-2 25.0 U/mL hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Reporting group title	APO-2 50.0 U/mL (FAS)
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Reporting group description:

Patients in the FAS who were administered APO-2 50.0 U/mL hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Reporting group title	Placebo (FAS)
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Reporting group description:

Patients in the FAS who were administered the placebo hydrogel formulation. The FAS included all randomized patients who received at least 1 dose of the IMP and had a measured wound area at Baseline and at Visit 6 or later.

Reporting group title	APO-2 12.5 U/mL (FAS posthoc subgroup)
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Reporting group description:

Patients in the FAS posthoc subgroup who were administered APO-2 12.5 U/mL hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Reporting group title	APO-2 25.0 U/mL (FAS posthoc subgroup)
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Reporting group description:

Patients in the FAS posthoc subgroup who were administered APO-2 25.0 U/mL hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Reporting group title	APO-2 50.0 U/mL (FAS posthoc subgroup)
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Reporting group description:

Patients in the FAS posthoc subgroup who were administered APO-2 50.0 U/mL hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Reporting group title	Placebo (FAS posthoc subgroup)
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Reporting group description:

Patients in the FAS posthoc subgroup who were administered the placebo hydrogel formulation. The FAS subgroup included all patients in the FAS with adjudicated wound areas ≥ 0.8 cm² at Visit 2. Patients

were randomized based on the investigator's assessment of wound area, which was required to be between 1 cm² (since protocol Version 4.0: 0.8 cm²) and 8 cm² in size. After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm² at Randomization according to the adjudicated, centrally assessed measurements, which were used for the primary endpoint analysis. A contributing factor for this discrepancy may have been that the protocol was not clear on how to account for keratotic tissue in the measurements. For this reason, a posthoc analysis was performed, in which the 28 patients were excluded from the analysis.

Primary: Percentage wound area reduction after 4 weeks treatment compared between groups (FAS)

End point title	Percentage wound area reduction after 4 weeks treatment compared between groups (FAS) ^[1]
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End point description:

Centrally adjudicated wound area measurements were used for the primary endpoint assessment. At completion of the study, photographic images of the patients' wounds, blinded to group assignment, were reviewed by 2 independent trained assessors, who acted as adjudicators in validating the wound area measurements.

End point type	Primary
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End point timeframe:

Reduction from Baseline to Visit 14, ie, after 4 weeks of IMP treatment

Notes:

[1] - 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: This endpoint only reports statistics for the arms that include the patients in the FAS. Statistics for the arms that include the patients in the FAS posthoc subgroup are reported separately.

End point values	APO-2 12.5 U/mL (FAS)	APO-2 25.0 U/mL (FAS)	APO-2 50.0 U/mL (FAS)	Placebo (FAS)
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	27	38	26	31
Units: percent				
arithmetic mean (standard deviation)	58.55 (± 45.58)	30.66 (± 113.57)	47.09 (± 59.93)	29.03 (± 136.82)

Statistical analyses

Statistical analysis title	APO-2 12.5 U/mL vs Placebo - FAS
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Statistical analysis description:

Analysis of variance (ANOVA) including the stratification factors country and wound area

Comparison groups	APO-2 12.5 U/mL (FAS) v Placebo (FAS)
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Number of subjects included in analysis	58
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Analysis specification	Pre-specified
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Analysis type	superiority
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P-value	= 0.5938 ^[2]
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Method	ANOVA
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Parameter estimate	Least squares mean difference
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Point estimate	28.57
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Confidence interval

level	95 %
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sides	2-sided
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lower limit	-36.09
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upper limit	93.22
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Notes:

[2] - 2-sided alpha = 0.05; Dunnett-adjusted

Statistical analysis title	APO-2 25.0 U/mL vs Placebo - FAS
Statistical analysis description: ANOVA including the stratification factors country and wound area	
Comparison groups	APO-2 25.0 U/mL (FAS) v Placebo (FAS)
Number of subjects included in analysis	69
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 1 [3]
Method	ANOVA
Parameter estimate	Least squares mean difference
Point estimate	0.85
Confidence interval	
level	95 %
sides	2-sided
lower limit	-58.46
upper limit	60.15

Notes:

[3] - 2-sided alpha = 0.05; Dunnett-adjusted

Statistical analysis title	APO-2 50.0 U/mL vs Placebo - FAS
Statistical analysis description: ANOVA including the stratification factors country and wound area	
Comparison groups	APO-2 50.0 U/mL (FAS) v Placebo (FAS)
Number of subjects included in analysis	57
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.8573 [4]
Method	ANOVA
Parameter estimate	Least squares mean difference
Point estimate	17.6
Confidence interval	
level	95 %
sides	2-sided
lower limit	-47.06
upper limit	82.25

Notes:

[4] - 2-sided alpha = 0.05; Dunnett-adjusted

Secondary: Proportion of patients with complete wound closure until Visit 17 (FAS)

End point title	Proportion of patients with complete wound closure until Visit 17 (FAS) ^[5]
End point description: Complete wound closure was defined as 100% re-epithelialization of the wound surface with the absence of drainage.	
End point type	Secondary
End point timeframe: Complete wound closure until Visit 17, ie, during the 12-week follow-up period	

Notes:

[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: This endpoint only reports statistics for the arms that include the patients in the FAS.

Statistics for the arms that include the patients in the FAS posthoc subgroup are reported separately.

End point values	APO-2 12.5 U/mL (FAS)	APO-2 25.0 U/mL (FAS)	APO-2 50.0 U/mL (FAS)	Placebo (FAS)
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	26	37	25	30
Units: patients	7	12	9	8

Statistical analyses

Statistical analysis title	APO-2 12.5 U/mL vs Placebo - FAS
Comparison groups	APO-2 12.5 U/mL (FAS) v Placebo (FAS)
Number of subjects included in analysis	56
Analysis specification	Pre-specified
Analysis type	superiority ^[6]
P-value	= 1 ^[7]
Method	Fisher exact

Notes:

[6] - 2-sided Fisher's exact test

[7] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 25.0 U/mL vs Placebo - FAS
Comparison groups	APO-2 25.0 U/mL (FAS) v Placebo (FAS)
Number of subjects included in analysis	67
Analysis specification	Pre-specified
Analysis type	superiority ^[8]
P-value	= 0.789 ^[9]
Method	Fisher exact

Notes:

[8] - 2-sided Fisher's exact test

[9] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 50.0 U/mL vs Placebo - FAS
Comparison groups	APO-2 50.0 U/mL (FAS) v Placebo (FAS)
Number of subjects included in analysis	55
Analysis specification	Pre-specified
Analysis type	superiority ^[10]
P-value	= 0.5616 ^[11]
Method	Fisher exact

Notes:

[10] - 2-sided Fisher's exact test

[11] - 2-sided alpha = 0.05

Post-hoc: Percentage wound area reduction after 4 weeks treatment compared between groups (FAS posthoc subgroup)

End point title	Percentage wound area reduction after 4 weeks treatment compared between groups (FAS posthoc subgroup) ^[12]
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End point description:

Centrally adjudicated wound area measurements were used for this endpoint assessment. At completion of the study, photographic images of the patients' wounds, blinded to group assignment, were reviewed by 2 independent trained assessors, who acted as adjudicators in validating the wound area measurements.

End point type | Post-hoc

End point timeframe:

Reduction from Baseline to Visit 14, ie, after 4 weeks of IMP treatment

Notes:

[12] - 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: This endpoint only reports statistics for the arms that include the patients in the FAS posthoc subgroup. Statistics for the arms that include the patients in the FAS are reported separately.

End point values	APO-2 12.5 U/mL (FAS posthoc subgroup)	APO-2 25.0 U/mL (FAS posthoc subgroup)	APO-2 50.0 U/mL (FAS posthoc subgroup)	Placebo (FAS posthoc subgroup)
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	23	27	21	23
Units: percent				
arithmetic mean (standard deviation)	55.40 (± 47.65)	46.05 (± 47.46)	41.63 (± 63.11)	25.07 (± 155.96)

Statistical analyses

Statistical analysis title | APO-2 12.5 U/mL vs Placebo - FAS subgroup

Statistical analysis description:

ANOVA including the stratification factors country and wound area

Comparison groups	APO-2 12.5 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	46
Analysis specification	Post-hoc
Analysis type	superiority
P-value	= 0.6177 [13]
Method	ANOVA
Parameter estimate	Least squares mean difference
Point estimate	27.78
Confidence interval	
level	95 %
sides	2-sided
lower limit	-37.6
upper limit	93.17

Notes:

[13] - 2-sided alpha = 0.05; Dunnett adjusted

Statistical analysis title | APO-2 25.0 U/mL vs Placebo - FAS subgroup

Statistical analysis description:

ANOVA including the stratification factors country and wound area

Comparison groups	APO-2 25.0 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
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Number of subjects included in analysis	50
Analysis specification	Post-hoc
Analysis type	superiority
P-value	= 0.6745 [14]
Method	ANOVA
Parameter estimate	Least squares mean difference
Point estimate	24.73
Confidence interval	
level	95 %
sides	2-sided
lower limit	-38.56
upper limit	88.03

Notes:

[14] - 2-sided alpha = 0.05; Dunnett adjusted

Statistical analysis title	APO-2 50.0 U/mL vs Placebo - FAS subgroup
Statistical analysis description: ANOVA including the stratification factors country and wound area	
Comparison groups	APO-2 50.0 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	44
Analysis specification	Post-hoc
Analysis type	superiority
P-value	= 0.8927 [15]
Method	ANOVA
Parameter estimate	Least squares mean difference
Point estimate	15.91
Confidence interval	
level	95 %
sides	2-sided
lower limit	-50.11
upper limit	81.93

Notes:

[15] - 2-sided alpha = 0.05; Dunnett adjusted

Post-hoc: Proportion of patients with complete wound closure until Visit 17 (FAS posthoc subgroup)

End point title	Proportion of patients with complete wound closure until Visit 17 (FAS posthoc subgroup) ^[16]
End point description: Complete wound closure was defined as 100% re-epithelialization of the wound surface with the absence of drainage.	
End point type	Post-hoc
End point timeframe: Complete wound closure until Visit 17, ie, during the 12-week follow-up period	

Notes:

[16] - 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: This endpoint only reports statistics for the arms that include the patients in the FAS posthoc subgroup. Statistics for the arms that include the patients in the FAS are reported separately.

End point values	APO-2 12.5 U/mL (FAS posthoc subgroup)	APO-2 25.0 U/mL (FAS posthoc subgroup)	APO-2 50.0 U/mL (FAS posthoc subgroup)	Placebo (FAS posthoc subgroup)
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	22	26	20	22
Units: patients	6	9	6	4

Statistical analyses

Statistical analysis title	APO-2 12.5 U/mL vs Placebo - FAS subgroup
Comparison groups	APO-2 12.5 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	44
Analysis specification	Post-hoc
Analysis type	superiority ^[17]
P-value	= 0.7205 ^[18]
Method	Fisher exact

Notes:

[17] - 2-sided Fisher's exact test

[18] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 25 U/mL vs Placebo - FAS subgroup
Comparison groups	APO-2 25.0 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	48
Analysis specification	Post-hoc
Analysis type	superiority ^[19]
P-value	= 0.3288 ^[20]
Method	Fisher exact

Notes:

[19] - 2-sided Fisher's exact test

[20] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 50 U/mL vs Placebo - FAS subgroup
Comparison groups	APO-2 50.0 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	42
Analysis specification	Post-hoc
Analysis type	superiority ^[21]
P-value	= 0.4769 ^[22]
Method	Fisher exact

Notes:

[21] - 2-sided Fisher's exact test

[22] - 2-sided alpha = 0.05

Post-hoc: Proportion of patients with complete wound closure until Visit 14 (FAS and FAS posthoc subgroup)

End point title	Proportion of patients with complete wound closure until Visit 14 (FAS and FAS posthoc subgroup)
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End point description:

Complete wound closure was defined as 100% re-epithelialization of the wound surface with the

absence of drainage.

End point type	Post-hoc
End point timeframe:	
Complete wound closure until Visit 14, ie, during the 4-week treatment	

End point values	APO-2 12.5 U/mL (FAS)	APO-2 25.0 U/mL (FAS)	APO-2 50.0 U/mL (FAS)	Placebo (FAS)
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	27	37	23	30
Units: patients	4	2	5	4

End point values	APO-2 12.5 U/mL (FAS posthoc subgroup)	APO-2 25.0 U/mL (FAS posthoc subgroup)	APO-2 50.0 U/mL (FAS posthoc subgroup)	Placebo (FAS posthoc subgroup)
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	23	26	20	22
Units: patients	3	1	3	1

Statistical analyses

Statistical analysis title	APO-2 12.5 U/mL vs Placebo - FAS
Comparison groups	APO-2 12.5 U/mL (FAS) v Placebo (FAS)
Number of subjects included in analysis	57
Analysis specification	Post-hoc
Analysis type	superiority ^[23]
P-value	= 1 ^[24]
Method	Fisher exact

Notes:

[23] - 2-sided Fisher's exact test

[24] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 25.0 U/mL vs Placebo - FAS
Comparison groups	APO-2 25.0 U/mL (FAS) v Placebo (FAS)
Number of subjects included in analysis	67
Analysis specification	Post-hoc
Analysis type	superiority ^[25]
P-value	= 0.396 ^[26]
Method	Fisher exact

Notes:

[25] - 2-sided Fisher's exact test

[26] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 50.0 U/mL vs Placebo - FAS
Comparison groups	APO-2 50.0 U/mL (FAS) v Placebo (FAS)

Number of subjects included in analysis	53
Analysis specification	Post-hoc
Analysis type	superiority ^[27]
P-value	= 0.478 ^[28]
Method	Fisher exact

Notes:

[27] - 2-sided Fisher's exact test

[28] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 12.5 U/mL vs Placebo - FAS subgroup
Comparison groups	APO-2 12.5 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	45
Analysis specification	Post-hoc
Analysis type	superiority ^[29]
P-value	= 0.6078 ^[30]
Method	Fisher exact

Notes:

[29] - 2-sided Fisher's exact test

[30] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 25.0 U/mL vs Placebo - FAS subgroup
Comparison groups	APO-2 25.0 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	48
Analysis specification	Post-hoc
Analysis type	superiority ^[31]
P-value	= 1 ^[32]
Method	Fisher exact

Notes:

[31] - 2-sided Fisher's exact test

[32] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 50.0 U/mL vs Placebo - FAS subgroup
Comparison groups	APO-2 50.0 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	42
Analysis specification	Post-hoc
Analysis type	superiority ^[33]
P-value	= 0.3327 ^[34]
Method	Fisher exact

Notes:

[33] - 2-sided Fisher's exact test

[34] - 2-sided alpha = 0.05

Post-hoc: Proportion of patients with a ≥80% reduction from Baseline in wound area at Visit 14 (FAS and FAS posthoc subgroup)

End point title	Proportion of patients with a ≥80% reduction from Baseline in wound area at Visit 14 (FAS and FAS posthoc subgroup)
End point description:	Proportion of patients with a ≥80% wound area reduction from Baseline at Visit 14
End point type	Post-hoc

End point timeframe:
at Visit 14 (after 4 weeks of treatment)

End point values	APO-2 12.5 U/mL (FAS)	APO-2 25.0 U/mL (FAS)	APO-2 50.0 U/mL (FAS)	Placebo (FAS)
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	27	37	23	30
Units: percent	9	10	11	6

End point values	APO-2 12.5 U/mL (FAS posthoc subgroup)	APO-2 25.0 U/mL (FAS posthoc subgroup)	APO-2 50.0 U/mL (FAS posthoc subgroup)	Placebo (FAS posthoc subgroup)
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	23	26	20	22
Units: percent	7	8	9	5

Statistical analyses

Statistical analysis title	APO-2 12.5 U/mL vs Placebo - FAS
Comparison groups	APO-2 12.5 U/mL (FAS) v Placebo (FAS)
Number of subjects included in analysis	57
Analysis specification	Post-hoc
Analysis type	superiority ^[35]
P-value	= 0.3675 ^[36]
Method	Fisher exact

Notes:

[35] - 2-sided Fisher's exact test

[36] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 25.0 U/mL vs Placebo - FAS
Comparison groups	APO-2 25.0 U/mL (FAS) v Placebo (FAS)
Number of subjects included in analysis	67
Analysis specification	Post-hoc
Analysis type	superiority ^[37]
P-value	= 0.5736 ^[38]
Method	Fisher exact

Notes:

[37] - 2-sided Fisher's exact test

[38] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 50.0 U/mL vs Placebo - FAS
Comparison groups	APO-2 50.0 U/mL (FAS) v Placebo (FAS)

Number of subjects included in analysis	53
Analysis specification	Post-hoc
Analysis type	superiority ^[39]
P-value	= 0.0412 ^[40]
Method	Fisher exact

Notes:

[39] - 2-sided Fisher's exact test

[40] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 12.5 U/mL vs Placebo - FAS subgroup
Comparison groups	APO-2 12.5 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	45
Analysis specification	Post-hoc
Analysis type	superiority ^[41]
P-value	= 0.7381 ^[42]
Method	Fisher exact

Notes:

[41] - 2-sided Fisher's exact test

[42] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 25.0 U/mL vs Placebo - FAS subgroup
Comparison groups	APO-2 25.0 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	48
Analysis specification	Post-hoc
Analysis type	superiority ^[43]
P-value	= 0.7456 ^[44]
Method	Fisher exact

Notes:

[43] - 2-sided Fisher's exact test

[44] - 2-sided alpha = 0.05

Statistical analysis title	APO-2 50.0 U/mL vs Placebo - FAS subgroup
Comparison groups	APO-2 50.0 U/mL (FAS posthoc subgroup) v Placebo (FAS posthoc subgroup)
Number of subjects included in analysis	42
Analysis specification	Post-hoc
Analysis type	superiority ^[45]
P-value	= 0.1917 ^[46]
Method	Fisher exact

Notes:

[45] - 2-sided Fisher's exact test

[46] - 2-sided alpha = 0.05

Adverse events

Adverse events information

Timeframe for reporting adverse events:

From the time of giving written informed consent until the end-of-the-study visit.

Assessment type	Systematic
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Dictionary used

Dictionary name	MedDRA
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Dictionary version	23.1
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Reporting groups

Reporting group title	APO-2 12.5 U/mL (SAF)
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Reporting group description:

Patients in the safety analysis set (SAF) who were administered APO-2 12.5 U/mL hydrogel formulation. The SAF included all randomized patients who received at least 1 dose of the IMP.

Reporting group title	APO-2 25.0 U/mL (SAF)
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Reporting group description:

Patients in the SAF who were administered APO-2 25.0 U/mL hydrogel formulation. The SAF included all randomized patients who received at least 1 dose of the IMP.

Reporting group title	APO-2 50.0 U/mL (SAF)
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Reporting group description:

Patients in the SAF who were administered APO-2 50.0 U/mL hydrogel formulation. The SAF included all randomized patients who received at least 1 dose of the IMP.

Reporting group title	Placebo (SAF)
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Reporting group description:

Patients in the SAF administered the placebo hydrogel formulation. The SAF includes all randomized patients who received at least 1 dose of the IMP.

Serious adverse events	APO-2 12.5 U/mL (SAF)	APO-2 25.0 U/mL (SAF)	APO-2 50.0 U/mL (SAF)
Total subjects affected by serious adverse events			
subjects affected / exposed	2 / 27 (7.41%)	3 / 38 (7.89%)	2 / 26 (7.69%)
number of deaths (all causes)	0	0	0
number of deaths resulting from adverse events	0	0	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)			
Adenocarcinoma of colon			
subjects affected / exposed	0 / 27 (0.00%)	1 / 38 (2.63%)	0 / 26 (0.00%)
occurrences causally related to treatment / all	0 / 0	0 / 1	0 / 0
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0
Vascular disorders			
Peripheral ischaemia			
subjects affected / exposed	0 / 27 (0.00%)	0 / 38 (0.00%)	0 / 26 (0.00%)
occurrences causally related to treatment / all	0 / 0	0 / 0	0 / 0
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0

Skin and subcutaneous tissue disorders			
Skin ulcer			
subjects affected / exposed	1 / 27 (3.70%)	0 / 38 (0.00%)	0 / 26 (0.00%)
occurrences causally related to treatment / all	0 / 1	0 / 0	0 / 0
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0
Infections and infestations			
Application site infection			
subjects affected / exposed	0 / 27 (0.00%)	1 / 38 (2.63%)	0 / 26 (0.00%)
occurrences causally related to treatment / all	0 / 0	0 / 1	0 / 0
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0
Cellulitis			
subjects affected / exposed	0 / 27 (0.00%)	0 / 38 (0.00%)	1 / 26 (3.85%)
occurrences causally related to treatment / all	0 / 0	0 / 0	0 / 1
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0
Diabetic foot infection			
subjects affected / exposed	0 / 27 (0.00%)	0 / 38 (0.00%)	1 / 26 (3.85%)
occurrences causally related to treatment / all	0 / 0	0 / 0	0 / 1
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0
Gangrene			
subjects affected / exposed	0 / 27 (0.00%)	1 / 38 (2.63%)	0 / 26 (0.00%)
occurrences causally related to treatment / all	0 / 0	0 / 2	0 / 0
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0
Infected skin ulcer			
subjects affected / exposed	1 / 27 (3.70%)	0 / 38 (0.00%)	0 / 26 (0.00%)
occurrences causally related to treatment / all	0 / 1	0 / 0	0 / 0
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0
Osteomyelitis			
subjects affected / exposed	0 / 27 (0.00%)	1 / 38 (2.63%)	0 / 26 (0.00%)
occurrences causally related to treatment / all	0 / 0	0 / 1	0 / 0
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0

Serious adverse events	Placebo (SAF)		
Total subjects affected by serious adverse events			
subjects affected / exposed	2 / 31 (6.45%)		
number of deaths (all causes)	0		

number of deaths resulting from adverse events	0		
Neoplasms benign, malignant and unspecified (incl cysts and polyps) Adenocarcinoma of colon subjects affected / exposed	0 / 31 (0.00%)		
occurrences causally related to treatment / all	0 / 0		
deaths causally related to treatment / all	0 / 0		
Vascular disorders Peripheral ischaemia subjects affected / exposed	1 / 31 (3.23%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Skin and subcutaneous tissue disorders Skin ulcer subjects affected / exposed	0 / 31 (0.00%)		
occurrences causally related to treatment / all	0 / 0		
deaths causally related to treatment / all	0 / 0		
Infections and infestations Application site infection subjects affected / exposed	0 / 31 (0.00%)		
occurrences causally related to treatment / all	0 / 0		
deaths causally related to treatment / all	0 / 0		
Cellulitis subjects affected / exposed	1 / 31 (3.23%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Diabetic foot infection subjects affected / exposed	0 / 31 (0.00%)		
occurrences causally related to treatment / all	0 / 0		
deaths causally related to treatment / all	0 / 0		
Gangrene subjects affected / exposed	0 / 31 (0.00%)		
occurrences causally related to treatment / all	0 / 0		
deaths causally related to treatment / all	0 / 0		
Infected skin ulcer			

subjects affected / exposed	0 / 31 (0.00%)		
occurrences causally related to treatment / all	0 / 0		
deaths causally related to treatment / all	0 / 0		
Osteomyelitis			
subjects affected / exposed	0 / 31 (0.00%)		
occurrences causally related to treatment / all	0 / 0		
deaths causally related to treatment / all	0 / 0		

Frequency threshold for reporting non-serious adverse events: 5 %

Non-serious adverse events	APO-2 12.5 U/mL (SAF)	APO-2 25.0 U/mL (SAF)	APO-2 50.0 U/mL (SAF)
Total subjects affected by non-serious adverse events			
subjects affected / exposed	7 / 27 (25.93%)	13 / 38 (34.21%)	8 / 26 (30.77%)
Investigations			
SARS-CoV-1 test positive			
subjects affected / exposed	0 / 27 (0.00%)	2 / 38 (5.26%)	0 / 26 (0.00%)
occurrences (all)	0	2	0
General disorders and administration site conditions			
Application site erythema			
subjects affected / exposed	0 / 27 (0.00%)	4 / 38 (10.53%)	0 / 26 (0.00%)
occurrences (all)	0	4	0
Application site reaction			
subjects affected / exposed	1 / 27 (3.70%)	2 / 38 (5.26%)	0 / 26 (0.00%)
occurrences (all)	1	3	0
Condition aggravated			
subjects affected / exposed	2 / 27 (7.41%)	2 / 38 (5.26%)	3 / 26 (11.54%)
occurrences (all)	2	2	4
Disease recurrence			
subjects affected / exposed	1 / 27 (3.70%)	3 / 38 (7.89%)	3 / 26 (11.54%)
occurrences (all)	2	3	3
Skin and subcutaneous tissue disorders			
Skin ulcer			
subjects affected / exposed	3 / 27 (11.11%)	3 / 38 (7.89%)	2 / 26 (7.69%)
occurrences (all)	3	3	2
Infections and infestations			

Application site infection subjects affected / exposed occurrences (all)	0 / 27 (0.00%) 0	3 / 38 (7.89%) 3	0 / 26 (0.00%) 0
Infected skin ulcer subjects affected / exposed occurrences (all)	0 / 27 (0.00%) 0	2 / 38 (5.26%) 3	1 / 26 (3.85%) 1

Non-serious adverse events	Placebo (SAF)		
Total subjects affected by non-serious adverse events subjects affected / exposed	7 / 31 (22.58%)		
Investigations SARS-CoV-1 test positive subjects affected / exposed occurrences (all)	1 / 31 (3.23%) 1		
General disorders and administration site conditions Application site erythema subjects affected / exposed occurrences (all) Application site reaction subjects affected / exposed occurrences (all) Condition aggravated subjects affected / exposed occurrences (all) Disease recurrence subjects affected / exposed occurrences (all)	1 / 31 (3.23%) 2 0 / 31 (0.00%) 0 3 / 31 (9.68%) 3 0 / 31 (0.00%) 0		
Skin and subcutaneous tissue disorders Skin ulcer subjects affected / exposed occurrences (all)	1 / 31 (3.23%) 1		
Infections and infestations Application site infection subjects affected / exposed occurrences (all) Infected skin ulcer	1 / 31 (3.23%) 1		

subjects affected / exposed	0 / 31 (0.00%)		
occurrences (all)	0		

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
06 November 2019	The screening phase was extended by 7 days. It was clarified that the minimum duration of a patient in the safety lead-in phase was 93 instead of 98 days, with a maximum of about 117 instead of 116 days. The description of the IMP and the planned wound assessments were updated. For the stratification by wound area, proportions of patients for each category were added. Details about the procedures to follow in case of occurrence and reporting of pregnancies and about the reporting of serious adverse events were added. Details about the neurological assessment of the foot were added.
25 June 2021	Inclusion criterion 1 was revised to add an upper age restriction of 70 years for sites in the Czech Republic. Inclusion criterion 8 was revised, ie, the ankle brachial index (ABI) at the leg with the treated wound was to be ≥ 0.9 (instead of between 0.7 and 1.3) with the lowest (not the highest) measured value being used as reference. The index wound duration described in exclusion criterion 3 was changed from >52 weeks to >3 years. In exclusion criterion 5, definitions for wound infection, osteomyelitis, and cellulitis were added. It was clarified that patients were to receive IMP treatment 3 times per week within 7 days. The risk benefit assessment was updated. It was added that biologically or chemically active dressings were not permitted during the study. Local adverse events (AEs) were defined. Visit windows were extended and it was clarified that treatment visits could be postponed.
09 May 2022	The upper age limit for patients in the Czech Republic was removed in inclusion criterion 1. Inclusion criterion 5 was changed to include patients with an estimated foot ulcer surface area ≥ 0.8 cm ² (formerly ≥ 1 cm ²) and ≤ 8 cm ² . In inclusion criterion 8, the ABI at the leg with the treated wound was defined to be ≥ 0.5 (instead of ≥ 0.9) and the toe pressure was to be >40 mmHg (instead of >50 mmHg) and it was added that patients with mild to moderate peripheral arterial disease (PAD) could be included. Exclusion criterion 5 was changed to exclude patients with a history of osteomyelitis during 8 weeks (instead of 6 months) before the screening visit. In exclusion criterion 7, "or current diagnosis of claudication" was deleted. A new exclusion criterion 7a was added to exclude certain patients with PAD (eg, patients with PAD of Fontaine Stage III or IV or acute peripheral artery occlusion). The minimum APO-2 dose was changed from 6.3 U to 2.5 U per wound and treatment. The stratification proportions for wound area were revised. The sample size of patients in the main study was changed from 120 to 108 randomized patients. History of PAD was added as assessment at Visit 1. It was clarified that a positive microbiological swab test result of the target wound alone was not to be considered a wound infection. The reporting procedure for local AEs was updated to include the involvement of the target wound (yes/no). The definition of the full analysis set was changed to include all patients of the safety analysis set with a measured wound area at Baseline and at Visit 6 or later. It was clarified that if the Visit 14 measurement of wound area was missing, the last available post-baseline measurement between Visit 6 and Visit 14 was to be used.

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 NU-GEL in the placebo had positive effects on wound healing. A treatment period of 4 weeks is too short. Unadjudicated wound assessment led to inclusion of patients with wounds <0.8cm². Synopsis of the study report is on <https://www.aposcience.at>

Notes: