Title page

Clinical Study Report

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)

MARSYAS II

EudraCT No.: 2018-001653-27

Investigational product:	APO-2
Clinical development phase:	IIa (first in human)
Indication:	Diabetic foot ulcer
Sponsor:	Aposcience AG Dresdner Straße 87/A 21 1200 Wien, Austria
Coordinating investigator:	Dr med Christiane Dreschl Allgemeines öffenliches Krankenhaus der Elisabethinen Klagenfurt Völkermarkter Straße 15-19 9020 Klagenfurt am Wörthersee Austria
Date of first patient enrolled:	11-Nov-2020
Date of last patient completed:	06-Dec-2023
Sponsor's signatory name:	Univ. Prof. Hendrik Jan Ankersmit, MD, MBA
Prepared by:	FGK Clinical Research GmbH Heimeranstr. 35 80339 München, Germany
Report version and date:	Final 1.0 (27-Nov-2024)

This study was performed in compliance with Good Clinical Practices (GCP) including the archiving of essential documents.

1 Synopsis

Name and address of sponsor: Aposcience AG**,** Dresdner Straße 87/A 21**,** 1200 Wien, Austria

Name of product: APO-2

Name of active ingredient: APOSEC^{TM}

Title of the study: 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)

Study registry: EudraCT no.: 2018-001653-27

Protocol number: MARSYAS II

Principal investigators and study sites:

4 sites in Austria, 1 site in Germany, 4 sites in Czech Republic, and 3 sites in Poland randomized patients in the study

Coordinating investigator: Dr med Christiane Dreschl, Allgemeines öffentliches Krankenhaus der Elisabethinen Klagenfurt, Völkermarkter Straße 15-19, 9020 Klagenfurt am Wörthersee, Austria

Publication (reference): Not applicable

Studied period:

11-Nov-2020 (first patient in) to 06-Dec-2023 (last patient out)

Reporting period: This report describes results of the final analysis.

Clinical phase: Phase IIa

Number of patients (total and for each treatment) planned and analyzed:

Planned: A minimum of 12 patients were to be randomized in the safety lead-in phase and 108 patients (27 patients per group) in the main study.

Analyzed:

122 patients were included in the full analysis set (FAS) and safety analysis set (SAF). A posthoc analysis was performed on a subgroup of patients with adjudicated wound areas ≥0.8 cm^2 at Visit 2. The rationale for the subgroup analysis is detailed in the section "Methodology" below. The following data sets were analyzed:

		Number of patients				
Population	**Data set**	**APO-2 12.5 U/mL**	**APO-2 25.0 U/mL**	**APO-2 50.0 U/mL**	**Placebo**	**All**
All patients enrolled	FAS	27	38	26	31	122
	SAF	27	38	26	31	122
	PPS	25	37	25	30	117
Patients with adjudicated wound areas ≥0.8 cm^2 at Visit 2 (posthoc analysis)	FAS	23	27	21	23	94
	SAF	23	27	21	23	94
	PPS	22	26	20	22	90

FAS = full analysis set, PPS = per-protocol set, SAF = safety analysis set.

Objectives and endpoints:

Primary objective: To determine the dose-response for clinical efficacy of APO-2 multiple dose administration in patients with diabetic foot ulcer at 3 different dose levels compared to placebo

Secondary objectives: Characterization of clinical safety and tolerability of APO-2, and the assessments of APO-2 efficacy on additional clinical endpoints at three different doses

Primary endpoint[1]: The percentage wound area reduction after 4 weeks treatment compared among groups

Secondary endpoints:

- >50% reduction in wound area after 4 weeks compared among groups
- Wound area change from Baseline at Visits 5, 8, 11, 14, 15, 16, 17 in each treatment group
- Proportion of patients with complete wound closure during 12-week follow-up period (100% re-epithelialization of the wound surface with the absence of drainage)
- Time to complete wound closure
- Recurrence rate of the ulcer during 12-week follow-up period after complete wound closure
- Clinical assessment of peripheral neuropathy at Baseline and at Visits 14 and 17 compared among groups and to Baseline
- Evaluation of wound pain by visual analogue scale (VAS) at Baseline, at every treatment visit, and at Visits 14, 15, 16, 17
- Evaluation of quality of life (QoL) using the Wound QoL questionnaire at Baseline and Visits 14 and 17 compared among groups
- Adverse events (AEs)
- Number of patients with local AEs with causal relationship to study medication or serious AEs (SAEs) with causal relationship to study medication

Further safety analyses: Vital signs, laboratory parameters, physical examination

Methodology:

This was a prospective, multicenter, randomized, double-blind, parallel group, placebo-controlled first in man Phase II study in patients with diabetic foot ulcer. The study consisted of a safety lead-in phase and a main study.

In the safety lead-in, a minimum of 12 eligible patients were randomly assigned in a 3:1 ratio to receive 25 U/mL APO-2 or placebo. The patients were treated for 4 weeks. Safety assessments included the monitoring of AEs, use of concomitant medications, and evaluation of wound area. When the last patient completed the 4-week treatment, a data and safety monitoring board (DSMB) reviewed the data during which time study enrollment was paused.

After the review, the DSMB was to give a recommendation as to whether or not to proceed with the main study as designed (ie, randomized, double-blind, parallel group). The DSMB could also recommend that additional patients be enrolled in the safety lead-in phase before initiating the main study, but this was ultimately not necessary. Upon a decision from the DSMB to proceed with the main study, enrollment resumed. Patients in the safety lead-in phase were

[1] The endpoints and variables are formulated as specified in the statistical analysis plan (SAP) Version 3.0, dated 13-Dec-2023. Further exploratory endpoints were added in the SAP.

followed for 8 weeks after the last administration of the investigational medicinal product (IMP).

In the main study, eligible patients were stratified by would area (at least 20% of patients needed to have a wound area >4 cm^2) and randomized to treatment with a low dose (12.5 U/mL), medium dose (25 U/mL), or high dose (50 U/mL) of APO-2 or with placebo. Patients were treated with the IMP 3 times per week for 4 weeks. The follow-up in the main study was 8 weeks.

In addition to the application of either APO-2 or placebo, standard of care was used for wound management throughout the study. The placebo contained a hydrogel (NU-GEL), which is not approved for the treatment of DFU. Consequently, this study compared APO-2 not against standard of care but against standard of care and NU-GEL.

The investigators used the eKare inSight tool to assess the wound size. In addition, a central assessment of the wound measurement was performed at the end of the study by independent and experienced assessors who were blinded to treatment.

Of note is that patients were randomized based on the investigator's assessment of wound area, while adjudicated measurements were used for the primary endpoint analysis. Wounds were required to be between 1 cm^2 (since protocol Version 4.0: 0.8 cm^2) and 8 cm^2 in size (see "Diagnosis and main eligibility criteria"). After unblinding and final data analysis, 28 patients were found to have wounds smaller than 0.8 cm^2 at randomization according to the adjudicated measurements. A potential limitation of the study is that the instructions in the protocol for measuring the wound size were unclear regarding the definition of wound margins, particularly in relation to hyperkeratosis. For this reason, a posthoc analysis of all study endpoints and variables was conducted based on only those patients with adjudicated wound areas ≥0.8 cm^2 at randomization (posthoc subgroup).

In addition to these analyses and the planned analyses on the per-protocol set (PPS), all efficacy analyses were repeated posthoc based on the PPS.

For an individual patient the study duration could be a minimum of 93 days and a maximum of 117 days including the allowed time windows.

Diagnosis and main eligibility criteria:

Key eligibility criteria were Type I or Type II diabetes with a glycosylated hemoglobin of ≤12% at or within 30 days before enrollment, a wound defined as diabetic foot ulcer present for ≥4 weeks, and a foot ulcer Wagner Grade I-II or ARMSTRONG Grade I-A or II-A.

The estimated foot ulcer surface area needed to be ≥1 cm^2 and ≤8 cm^2 as measured at randomization (Visit 2) by the investigator. With protocol Version 4.0, the lower limit of the required ulcer size was decreased to ≥0.8 cm^2.

Patients had to be between 18 and 80 years old and give their written informed consent before study start. Local protocol Version 2.2 for the Czech Republic introduced an upper age limit of 70 years. This change was added in global protocol Version 3.0 for sites in the Czech Republic only, but was removed again in protocol Version 4.0. Women of childbearing potential had to use adequate birth control during the study.

With protocol Version 4.0, patients with mild to moderate peripheral arterial disease (PAD) were allowed to be included in the study. The criteria for arterial blood perfusion were revised accordingly. A new exclusion criterion was added to exclude certain patients with PAD (eg, patients with PAD of Fontaine Stage III or IV or with acute peripheral artery occlusion).

Patients were to be excluded in case of a major uncontrolled medical disorder, clinical evidence of ulcer bed infection, requirement for intravenous antibiotic treatment of the index wound,

evidence of osteomyelitis, cellulitis, or infection, or significant disease that could impact the study.

Test product, dose, mode of administration, batch no.:

APO-2 (100 U APOSEC™ dissolved in 0.5 mL of 0.9% saline solution) diluted with 0.9% saline solution and mixed with NU-GEL up to a final volume of 4 mL; doses: 12.5 U/mL, 25 U/mL, or 50 U/mL APO-2, administered topically; batch no.: A000920399180, A000921399276, A000921399300, A000921399310, A000922399400

Reference product, dose, mode of administration, batch no.:

Placebo (culture medium processed as APOSEC™) diluted with 0.9% saline solution and mixed with NU-GEL; administered topically; batch no.: A000920399190, A000921399277, A000921399290, A000922399430

Duration of treatment: 4 weeks

Duration of follow-up: 8 weeks

Statistical methods:

The primary endpoint was analyzed confirmatory using an analysis of variance, including a Dunnett multiple comparison procedure to test each of the active doses against placebo based on the full analysis set. The stratification factors country and wound area were included in the analysis in order to account for the stratified randomization.

Secondary endpoints were analyzed descriptively. Secondary endpoints related to proportions of patients were additionally analyzed using a Fisher's exact test to test differences between treatment groups. Time to complete wound closure was analyzed using Kaplan-Meier estimates.

Further posthoc analyses were performed, such as ≥80% reduction from Baseline in wound area at Visits 14 and 17, complete wound closure until Visit 14, and graphical presentations of % reduction in wound area from Baseline over time.

Additionally, due to the differences between wound area measurements from the investigators and those centrally assessed, all analyses planned in the statistical analysis plan including the posthoc analyses described above were repeated based on patients with adjudicated wound areas of ≥0.8 cm^2 at randomization (posthoc analysis).

All efficacy analyses were additionally also repeated based on the PPS.

SUMMARY - CONCLUSIONS

Patient disposition

A total of 159 patients were enrolled in the study, 122 of whom were randomized and treated. 50 patients were treated under protocol Versions 2.0, 2.2, or 3.0. In August 2022, protocol Version 4.0 became valid, in which the lower wound area limit was reduced from 1.0 to 0.8 cm^2. A total of 72 patients were treated under protocol Version 4.0.

Of the 94 patients with adjudicated wound areas ≥0.8 cm^2 at Visit 2 (posthoc analysis), 36 patients were treated under protocol Versions 2.0, 2.2, or 3.0 and 58 patients were treated under protocol Version 4.0.

The study was completed by all but 3 patients, who terminated prematurely due to AEs. These 3 patients were in the subgroup of patients with adjudicated wound areas ≥0.8 cm^2 at Visit 2.

Main analysis - all patients

Demographics and baseline characteristics

The study population consisted of 85% men and 15% women. The mean ± SD age of the patients was 61.9 ± 10.3 years (range: 32 to 79 years).

Extent of exposure

Between 84% and 90% of patients in the treatment groups received the planned 12 IMP applications. The remaining patients received between 6 and 11 IMP applications. The mean total amount of IMP that was applied in the treatment groups ranged from 7.5 mL to 9.7 mL.

Efficacy results (FAS)

Primary endpoint

After 4 weeks of treatment, the APO-2 12.5 and 50 U/mL groups showed a higher mean and median % reduction in wound area than the placebo group, while the APO-2 25 U/mL group showed a similar reduction. The observed differences to placebo were not statistically significant (p-values >0.05).

Primary endpoint: % reduction in wound area from Baseline to Visit 14 (FAS, N = 122)

	APO-2 12.5 U/mL N = 27	APO-2 25.0 U/mL N = 38	APO-2 50.0 U/mL N = 26	Placebo N = 31
Mean (SD)	58.55 (45.58)	30.66 (113.57)	47.09 (59.93)	29.03 (136.82)
Median	66.70	60.00	74.35	60.00
Analysis of variance (confirmatory analysis)				
Diff. to pbo (LS means)	28.57	0.85	17.60	
[95% CI][a]	[-36.09; 93.22]	[-58.46; 60.15]	[-47.06; 82.25]	
P-value[a,b]	0.5938	1.0000	0.8573	
Van Elteren test (exploratory analysis)				
Diff. to pbo (medians)	6.70[c]	0.00[d]	14.35[c]	
P-value[b]	0.2192	0.8650	0.2861	

The stratification factors country and wound area were included in the analyses.
[a] Dunnett-adjusted. [b] 2-sided alpha = 0.05. [c] Including 55 patients. [d] Including 69 patients.
CI = confidence interval, diff. = difference, FAS = full analysis set, LS = least squares, N = number of patients in data set, pbo = placebo.

Secondary endpoints

Secondary endpoints were tested exploratory (2-sided alpha = 0.05) and are described below.

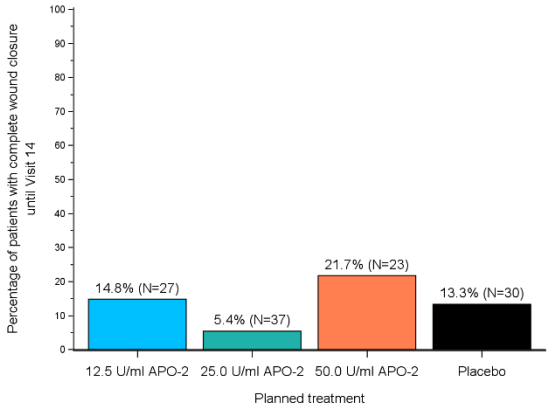
Patients with a >50% reduction in wound area, ulcer recurrence rate, and change in wound area (FAS, N = 122)

			APO-2 12.5 U/mL N = 27	APO-2 25.0 U/mL N = 38	APO-2 50.0 U/mL N = 26	Placebo N = 31
Patients with						
>50% reduction in wound area at V14		[n] N (%)	[27] 19 (70.4)	[37] 21 (56.8)	[23] 15 (65.2)	[30] 18 (60.0)
		P-value[a]	0.5790	0.8088	0.7794	
ulcer recurrence (12 weeks)		[n] N (%)	[6] 1 (16.7)	[7] 3 (42.9)	[8] 4 (50.0)	[6] 4 (66.7)
		P-value[a]	0.2424	0.5921	0.6270	
Wound area [cm²]	BL	n	27	38	26	31
		Mean (SD)	1.76 (1.18)	1.86 (1.75)	2.04 (1.40)	1.81 (1.68)
		Median	1.40	1.15	1.85	1.30
	CFB V14	n	27	37	25	30
		Mean (SD)	-0.91 (1.04)	-0.65 (1.09)	-0.93 (1.69)	-1.02 (1.07)
		Median	-1.00	-0.50	-0.80	-0.75

[a] 2-sided Fisher's exact test (2-sided alpha = 0.05), APO-2 dose group versus placebo.
BL = Baseline, CFB = change from Baseline, FAS = full analysis set, N = number of patients, n = number of patients analyzed, V = Visit.

Complete wound closure until Visit 14 was achieved in a higher percentage of patients in the APO-2 12.5 and 50 U/mL groups than in the placebo group (posthoc analysis).

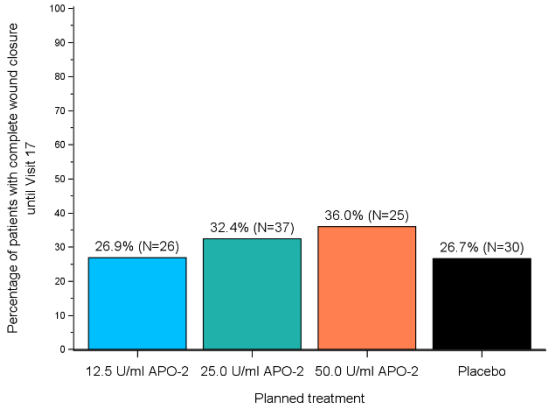
Complete wound closure until Visit 14 (FAS, N = 122; posthoc analysis)



FAS = full analysis set, N = number of patients in data set.

Complete wound closure until Visit 17 was achieved in a higher percentage of patients in the APO-2 25.0 and 50 U/mL groups than in the placebo group.

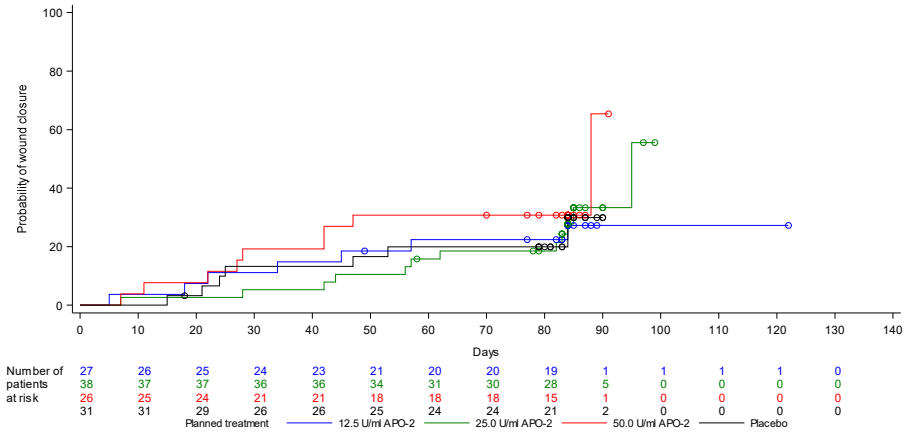
Complete wound closure until Visit 17 (FAS, N = 122)



FAS = full analysis set, N = number of patients in data set.

The median times to complete wound closure were 95 days in the APO-2 25 U/mL group and 88 days in the 50 U/mL group. The median could not be calculated in the other treatment groups. Kaplan Maier curves of the probability of wound closure until Visit 14 are shown in the figure below.

Probability of wound closure during 12-week follow-up (FAS, N = 122)



FAS = full analysis set, N = number of patients in data set. Posthoc analysis.

Wound assessments did not reveal a clinically relevant trend over time. The proportion of patients experiencing new sensation in the monofilament test at Visits 14 and/or 17 did not markedly differ between treatment groups. In the tuning fork test of the ulcerous foot, only a few patients in the APO-2 groups transitioned from feeling no vibration at Baseline to feeling vibration at Visits 14 and/or 17, and no patients in the placebo group.

The median baseline VAS scores for wound pain ranged from 4.0 to 11.5 mm. At Visit 14, the median change was -1.0 to 0.0 mm in the APO-2 groups compared to -2.5 mm in the placebo group. At Visit 17, the median change was -1.5 to 0.0 mm in the APO-2 groups compared to -2.0 mm in the placebo group. The median baseline Wound-QoL global scores ranged from 1.38 to 1.53. The APO-2 groups showed a median decrease of -0.24 to -0.06, compared to -0.27 in the placebo group at Visit 14, and of -0.35 to -0.06, compared to -0.24 at Visit 17.

A ≥80% reduction in wound area at Visit 14 was observed in a higher percentage of patients in the APO-2 12.5, 25, and 50 U/mL groups than in the placebo group (33%, 27%, and 48%,

respectively, vs 20%). The difference between the APO-2 50 U/mL group and the placebo group was significant (p-value: 0.0412, posthoc analysis).

Efficacy results (PPS)

In total, 5 patients (2 patients in the 12.5 U/mL APO-2 group, and 1 patient each in the other APO-2 groups and the placebo group) were excluded from the PPS due to major protocol deviations. Thus, 117 patients were included the PPS.

Primary endpoint

After 4 weeks of treatment, the APO-2 12.5 U/mL group showed a slightly higher mean and median % reduction in wound area than the placebo group. In all APO-2 groups, the observed differences to placebo were not statistically significant (p-values >0.05).

Primary endpoint: % reduction in wound area from Baseline to Visit 14 (PPS, N = 117)

	APO-2 12.5 U/mL N = 25	APO-2 25.0 U/mL N = 37	APO-2 50.0 U/mL N = 25	Placebo N = 30
Mean (SD)	59.64 (46.85)	31.49 (115.02)	47.09 (61.17)	52.50 (41.29)
Median	66.70	60.00	75.00	61.60
Analysis of variance				
Diff. to pbo (LS means)	4.27	-21.00	-7.48	
[95% CI][a]	[-46.52; 55.06]	[-66.72; 24.73]	[-57.74; 42.78]	
P-value[a,b]	0.9946	0.5656	0.9716	
Van Elteren test				
Diff. to pbo (medians)	5.10[c]	-1.60[d]	13.40[e]	
P-value[b]	0.1873	0.8335	0.4133	

The stratification factors country and wound area were included in the analyses.
[a] Dunnett-adjusted. [b] 2-sided alpha = 0.05. [c] Including 52 patients. [d] Including 67 patients. [e] Including 53 patients.
CI = confidence interval, diff. = difference, LS = least squares, N = number of patients in data set, pbo = placebo, PPS = per-protocol set.

Secondary endpoints (PPS, posthoc analyses)

Secondary endpoints are described below. All results in the PPS were in line with results in the FAS.

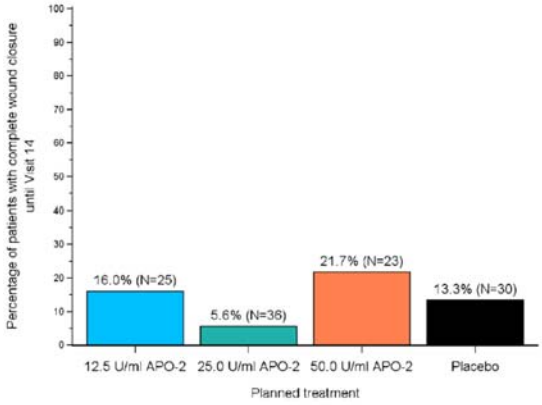
Patients with a >50% reduction in wound area, ulcer recurrence rate, and change in wound area (PPS, N = 117)

			APO-2 12.5 U/mL N = 25	APO-2 25.0 U/mL N = 37	APO-2 50.0 U/mL N = 25	Placebo N = 30
Patients with						
>50% reduction in wound area at V14		[n] N (%)	[25] 18 (72.0)	[36] 21 (58.3)	[23] 15 (65.2)	[30] 18 (60.0)
		P-value[a]	0.4040	1.0000	0.7794	
ulcer recurrence (12 weeks)		[n] N (%)	[6] 1 (16.7)	[7] 3 (42.9)	[8] 4 (50.0)	[6] 4 (66.7)
		P-value[a]	0.2424	0.5921	0.6270	
Wound area [cm^2]	BL	n	25	37	25	30
		Mean (SD)	1.70 (1.08)	1.86 (1.77)	1.98 (1.40)	1.85 (1.70)
		Median	1.40	1.10	1.80	1.30
	CFB V14	n	25	36	23	30
		Mean (SD)	-0.93 (1.08)	-0.67 (1.10)	-0.93 (1.69)	-1.02 (1.07)
		Median	-1.00	-0.55	-0.80	-0.75

[a] 2-sided Fisher's exact test (2-sided alpha = 0.05), APO-2 dose group versus placebo.
BL = Baseline, CFB = change from Baseline, N = number of patients, n = number of patients analyzed, PPS = per-protocol set, V = Visit. Posthoc analysis.

Complete wound closure until Visit 14 was achieved in a higher percentage of patients in the APO-2 12.5 and 50 U/mL groups than in the placebo group.

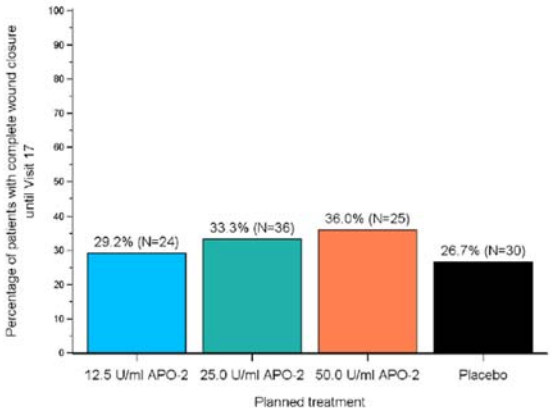
Complete wound closure until Visit 14 (PPS, N = 117)



N = number of patients in data set, PPS = per-protocol set. Posthoc analysis.

Complete wound closure until Visit 17 was achieved in a higher percentage of patients in all APO-2 groups than in the placebo group.

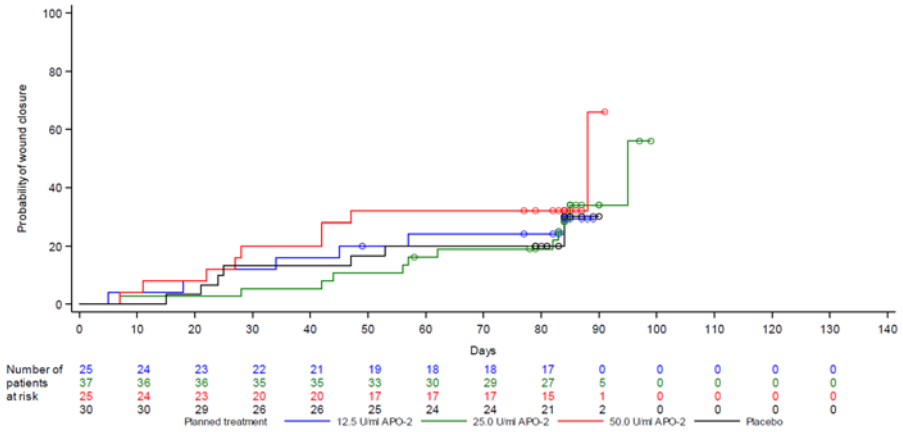
Complete wound closure until Visit 17 (PPS, N = 117)



N = number of patients in data set, PPS = per-protocol set. Posthoc analysis.

The median times to complete wound closure were 95 days in the APO-2 25 U/mL group and 88 days in the 50 U/mL group. The median time could not be calculated in the other treatment groups. Kaplan Maier curves of the probability of wound closure until Visit 14 are shown in the figure below.

Probability of wound closure during 12-week follow-up (PPS, N = 117)



N = number of patients in data set, PPS = per-protocol set. Posthoc analysis.

Further secondary endpoint results were in line with results observed in the FAS.

Safety results

TEAEs occurred in 35-47% of patients in the APO-2 groups with no dose-relationship and in 45% of patients in the placebo group. Common TEAEs included application site erythema, condition aggravated, disease recurrence, application site infection, and skin ulcer.

Most TEAEs were mild to moderate with only 5 TEAEs being severe. About half of the TEAEs were ongoing at the end of the observation.

Only 1 TEAE, application site reaction, was considered to be possibly related to the IMP by the investigator.

No deaths occurred in the study. A total of 11 SAEs, all considered unrelated to the IMP, were reported in 9 patients. The frequency of SAEs was similar in the treatment groups (7-8%). In 1 patient each in the APO-2 groups and 2 patients in the placebo group, treatment was prematurely discontinued or temporarily suspended because of a TEAE.

The clinical laboratory and vital sign measurements did not reveal any safety issues of treatment with APO-2.

Posthoc analyses in patients with adjudicated wound areas ≥0.8 cm^2 at Visit 2

In a posthoc analysis, only patients with adjudicated wound areas ≥0.8 cm^2 at Visit 2 were included. This subpopulation included 94 patients, ie, 28 patients with adjudicated wound areas <0.8 cm^2 at Visit 2 were excluded from the 122 patients in the FAS.

Demographics and baseline characteristics (FAS posthoc subgroup)

The subpopulation consisted of 84% men and 16% women. The mean ± SD age of the patients was 61.8 ± 10.5 years (range: 32 to 79 years).

Extent of exposure (FAS posthoc subgroup)

Between 86% and 91% of patients in the treatment groups received the planned 12 IMP applications. The remaining patients received between 6 and 11 IMP applications. The mean total amount of IMP that was applied in the treatment groups ranged from 7.8 mL to 12.1 mL.

Efficacy results (FAS posthoc subgroup)

Primary endpoint (FAS posthoc subgroup)

All APO-2 groups showed a higher mean % reduction in wound area than the placebo group after 4 weeks of treatment. The observed differences to placebo were not statistically significant (p-values >0.05).

Primary endpoint: % reduction in wound area from Baseline to Visit 14 (patients in the FAS with adjudicated wound areas ≥0.8 cm² at Visit 2, N = 94; posthoc analysis)

	APO-2 12.5 U/mL N = 23	APO-2 25.0 U/mL N = 27	APO-2 50.0 U/mL N = 21	Placebo N = 23
Mean (SD)	55.40 (47.65)	46.05 (47.46)	41.63 (63.11)	25.07 (155.96)
Median	63.60	60.70	73.70	63.20
Analysis of variance				
Diff. to pbo (LS means) [95% CI][a]	27.78 [-37.60; 93.17]	24.73 [-38.56; 88.03]	15.91 [-50.11; 81.93]	
P-value[a,b]	0.6177	0.6745	0.8927	
Van Elteren test				
Diff. to pbo (medians)	0.400	-2.500	10.500	
P-value[b]	0.5411	0.9213	0.3963	

The stratification factors country and wound area were included in the analyses.
[a] Dunnett-adjusted. [b] 2-sided alpha = 0.05. [c] Including 55 patients. [d] Including 69 patients.
CI = confidence interval, diff. = difference, FAS = full analysis set, LS = least squares, N = number of patients in data set, pbo = placebo.

Secondary endpoints (FAS posthoc subgroup)

Results of secondary endpoints are shown below.

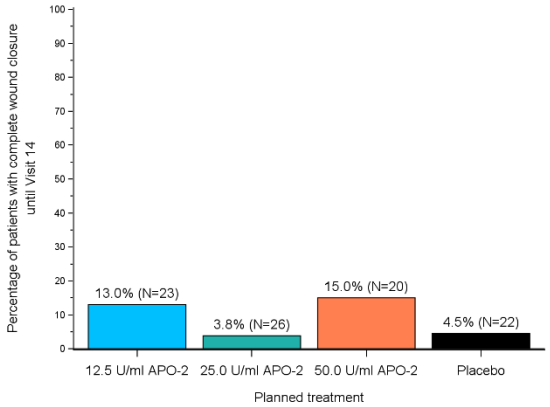
Patients with a >50% reduction in wound area, ulcer recurrence rate, and change in wound area (patients in the FAS with adjudicated wound areas ≥0.8 cm² at Visit 2, N = 94; posthoc analysis)

			APO-2 12.5 U/mL N = 23	APO-2 25.0 U/mL N = 27	APO-2 50.0 U/mL N = 21	Placebo N = 23
Patients with >50% reduction in wound area at V14		[n] N (%)	[23] 16 (69.6)	[26] 15 (57.7)	[20] 12 (60.0)	[22] 14 (63.6)
		P-value[a]	0.7575	0.7711	1.0000	
ulcer recurrence (12 weeks)		[n] N (%)	[5] 1 (20.0)	[5] 1 (20.0)	[5] 1 (20.0)	[2] 1 (50.0)
		P-value[a]	1.0000	1.0000	1.0000	
Wound area [cm²]	BL	n	23	27	21	23
		Mean (SD)	1.99 (1.12)	2.41 (1.80)	2.44 (1.26)	2.29 (1.71)
		Median	1.50	1.90	2.10	1.90
	CFB V14	n	23	26	20	22
		Mean (SD)	-1.02 (1.09)	-0.83 (1.25)	-1.03 (1.79)	-1.30 (1.12)
		Median	-1.00	-0.75	-1.00	-1.10

[a] 2-sided Fisher's exact test (2-sided alpha = 0.05), APO-2 dose group versus placebo.
BL = Baseline, CFB = change from Baseline, FAS = full analysis set, N = number of patients, n = number of patients analyzed, V = Visit.

Complete wound closure until Visit 14 was achieved in a higher percentage of patients in the APO-2 12.5 and 50 U/mL groups than in the placebo group.

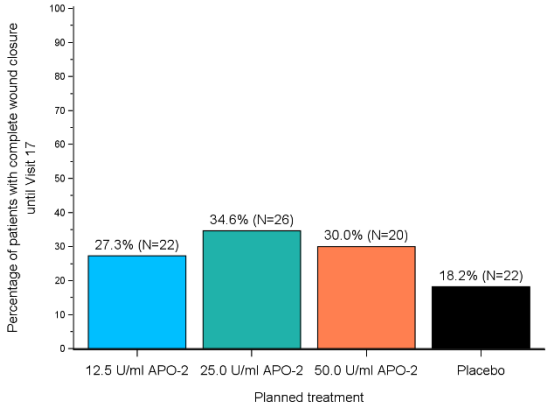
Complete wound closure until Visit 14 (patients in the FAS with adjudicated wound areas ≥0.8 cm^2 at Visit 2, N = 94; posthoc analysis)



FAS = full analysis set, N = number of patients.

Complete wound closure until Visit 17 was achieved in a higher percentage of patients in all APO-2 groups than in the placebo group.

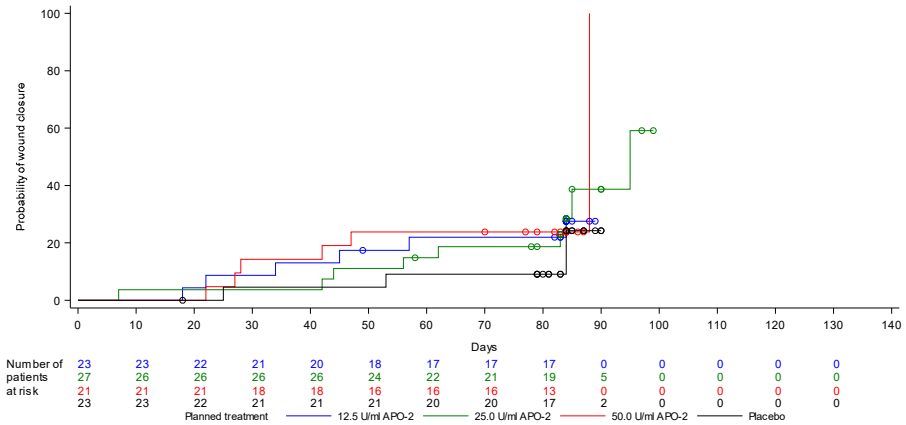
Complete wound closure until Visit 17 (patients in the FAS with adjudicated wound areas ≥0.8 cm^2 at Visit 2, N = 94; posthoc analysis)



FAS = full analysis set, N = number of patients.

The median times to complete wound closure were 95 days in the APO-2 25 U/mL group and 88 days in the 50 U/mL group. The median could not be calculated in the other treatment groups. Kaplan Maier curves of the probability of wound closure until Visit 14 are shown in the figure below.

Probability of wound closure during 12-week follow-up (patients in the FAS with adjudicated wound areas ≥0.8 cm^2 at Visit 2, N = 94; posthoc analysis)



FAS = full analysis set, N = number of patients.

Wound assessments did not reveal a clinically relevant trend over time. The proportion of patients experiencing new sensation in the monofilament test at Visits 14 and/or 17 did not markedly differ between treatment groups. In the tuning fork test of the ulcerous foot, only a few patients in the APO-2 groups transitioned from feeling no vibration at Baseline to feeling vibration at Visits 14 and/or 17, and no patients in the placebo group.

The median baseline VAS scores for wound pain ranged from 4.0 to 10.0 mm. At Visit 14, the median change was -1.5 to 0.0 mm in the APO-2 groups compared to -1.0 mm in the placebo group. At Visit 17, the median change was -2.0 to 0.0 mm in the APO-2 groups compared to -0.5 mm in the placebo group. The median baseline Wound-QoL global scores ranged from 1.29 to 1.71. The APO-2 groups showed a median decrease of -0.33 to -0.06, compared to -0.35 in the placebo group at Visit 14, and of -0.53 to -0.06, compared to -0.29 at Visit 17.

A ≥80% reduction in wound area at Visit 14 was observed in a higher percentage of patients in the APO-2 12.5, 25, and 50 U/mL groups (30%, 31%, and 45%, respectively) than in the placebo group (23%).

Efficacy results (PPS posthoc subgroup)

The PPS posthoc subgroup included 90 patients, ie, 4 patients of the FAS posthoc subgroup had major protocol deviations and were excluded from the PPS posthoc subgroup.

Primary endpoint

APO-2 groups showed a similar or smaller mean % reduction in wound area as compared to the placebo group after 4 weeks of treatment. The observed differences in all APO-2 groups to placebo were not statistically significant (p-values >0.05).

Primary endpoint: % reduction in wound area from Baseline to Visit 14 (patients in the PPS with adjudicated wound areas ≥0.8 cm^2 at Visit 2, N = 90; posthoc analysis)

	APO-2 12.5 U/mL N = 22	APO-2 25.0 U/mL N= 26	APO-2 50.0 U/mL N = 20	Placebo N = 22
Mean (SD)	56.86 (48.24)	47.82 (47.48)	41.36 (64.74)	56.89 (32.90)
Median	65.15	65.75	74.35	64.25
Analysis of variance				
Diff. to pbo (LS means) [95% CI][a]	-3.02 [-38.96; 32.91]	-3.98 [-38.47; 30.51]	-17.98 [-54.29; 18.33]	
P-value[a,b]	0.9944	0.9859	0.5025	
Van Elteren test				
Diff. to pbo (medians)	0.900[c]	1.500[d]	10.100[e]	
P-value[b]	0.5293	0.9155	0.6814	

The stratification factors country and wound area were included in the analyses.
[a] Dunnett-adjusted. [b] 2-sided alpha = 0.05. [c] Including 42 patients. [d] Including 44 patients. [e] Including 41 patients.
CI = confidence interval, diff. = difference, LS = least squares, N = number of patients in the data set, pbo = placebo, PPS = per-protocol set.

Secondary endpoints (PPS posthoc subgroup)

Results of secondary endpoints are shown below.

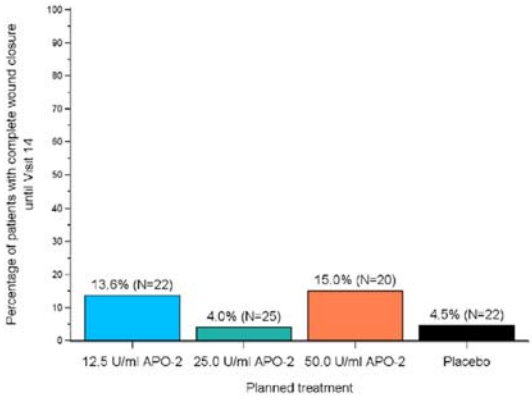
Patients with a >50% reduction in wound area, ulcer recurrence rate, and change in wound area (patients in the PPS with adjudicated wound areas ≥0.8 cm^2 at Visit 2, N = 90; posthoc analysis)

			APO-2 12.5 U/mL N = 22	APO-2 25.0 U/mL N= 26	APO-2 50.0 U/mL N = 20	Placebo N = 22
Patients with >50% reduction in wound area at V14		[n] N (%)	[22] 16 (72.7)	[25] 15 (60.0)	[20] 12 (60.0)	[22] 14 (63.6)
		P-value[a]	0.7470	1.0000	1.0000	
ulcer recurrence (12 weeks)		[n] N (%)	[5] 1 (20.0)	[5] 1 (20.0)	[5] 1 (20.0)	[2] 1 (50.0)
		P-value[a]	1.0000	1.0000	1.0000	
Wound area [cm^2]	BL	n	22	26	20	22
		Mean (SD)	1.88 (1.02)	2.43 (1.84)	2.39 (1.27)	2.36 (1.72)
		Median	1.50	1.70	2.10	1.95
	CFB V14	n	22	25	20	22
		Mean (SD)	-1.02 (1.12)	-0.86 (1.26)	-1.03 (1.79)	-1.30 (1.12)
		Median	-1.00	-0.80	-1.00	-1.10

[a] 2-sided Fisher's exact test (2-sided alpha = 0.05), APO-2 dose group versus placebo.
BL = Baseline, CFB = change from Baseline, N = number of patients, n = number of patients analyzed, PPS = per-protocol set, V = Visit.

Complete wound closure until Visit 14 was achieved in a higher percentage of patients in the APO-2 12.5 and 50 U/mL groups than in the placebo group.

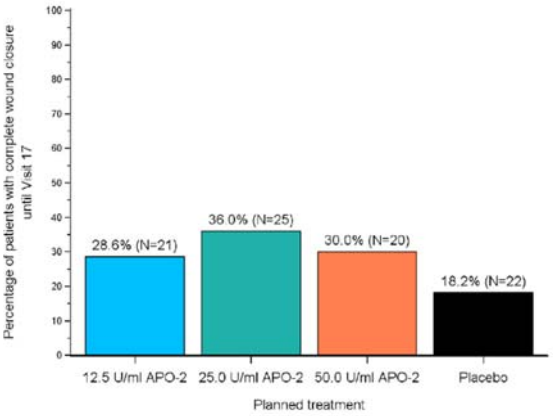
Complete wound closure until Visit 14 (patients in the PPS with adjudicated wound areas ≥0.8 cm^2 at Visit 2, N = 90; posthoc analysis)



N = number of patients, PPS = per-protocol set.

Complete wound closure until Visit 17 was achieved in a higher percentage of patients in all APO-2 groups than in the placebo group.

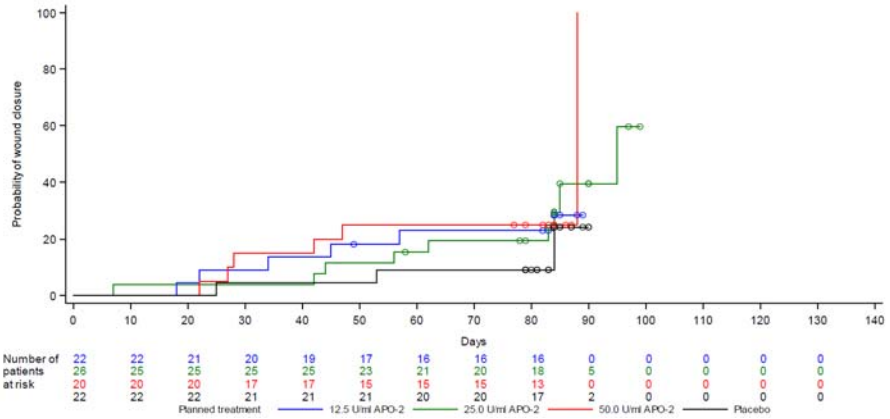
Complete wound closure until Visit 17 (patients in the PPS with adjudicated wound areas ≥0.8 cm^2 at Visit 2, N = 90; posthoc analysis)



N = number of patients, PPS = per-protocol set.

The median times to complete wound closure were 95 days in the APO-2 25 U/mL group and 88 days in the 50 U/mL group. The median time could not be calculated in the other treatment groups. Kaplan Maier curves of the probability of wound closure until Visit 14 are shown in the figure below.

Probability of wound closure during 12-week follow-up (patients in the PPS with adjudicated wound areas ≥0.8 cm^2 at Visit 2, N = 90; posthoc analysis)



N = number of patients, PPS = per-protocol set.

Further secondary endpoint results were also in line with results reported for the FAS subgroup.

Safety results (posthoc subgroup)

TEAEs occurred in 33-44% of patients in the APO-2 groups with no dose-relationship and in 48% of patients in the placebo group. Common TEAEs included condition aggravated, disease recurrence, application site infection, and skin ulcer.

Most TEAEs were mild to moderate with only 5 TEAEs being severe. Some TEAEs were ongoing at the end of the observation.

Only 1 TEAE, application site reaction, was considered to be related to the IMP by the investigator.

No deaths occurred. A total of 10 SAEs, all considered unrelated to the IMP, were reported in 8 patients. The frequency of SAEs was similar in the treatment groups (7-10%). In 1 patient each in the APO-2 25 and 50 U/mL groups and in 1 patient in the placebo group, treatment was prematurely discontinued because of a TEAE.

The clinical laboratory and vital sign measurements did not reveal any safety issues of treatment with APO-2.

Conclusions:

- The study results demonstrated that APO-2 has the potential to increase wound healing in patients with DFU. Posthoc analyses in only those patients with adjudicated wound areas ≥0.8 cm^2 at Visit 2 confirmed the positive trend observed in the overall population.
- Mean reductions in wound areas after 4 weeks (Visit 14) in the low (59%) and high (47%) APO-2 dose groups were numerically higher than in the placebo group (29%) in the overall population.
- A higher percentage of patients in all APO-2 groups achieved a ≥80% reduction in wound area after 4 weeks (Visit 14) compared to the placebo group, with a significant difference in the APO-2 50 U/mL group (48% vs 20%, posthoc analysis). Similar, non-significant differences between APO-2 and placebo were observed in patients with adjudicated wound areas ≥0.8 cm^2 at Visit 2.

- Complete wound closure rates until Visit 14 (Week 4) were higher in the low and high dose APO-2 groups than in the placebo group, in both the overall population and patients with adjudicated wound areas ≥0.8 cm^2 at Visit 2.
- Patients in the medium and high dose APO-2 groups had higher wound closure rates until Visit 17 (Week 12) than in the placebo group. In patients with adjudicated wound areas ≥0.8 cm^2 at Visit 2, all APO-2 groups showed higher wound closure rates, especially the APO-2 25.0 U/mL group with a wound closure rate almost twice as high as that in the placebo group (35% vs 18%).
- Higher than expected healing rates were observed in the placebo group in this study. It can be assumed that the NU-GEL compound of the placebo has already positive effects on wound healing of (lower grade) diabetic foot ulcers (Dumville et al., 2013)[2] and cannot be considered an effectless placebo, but there was no other way of proper blinding of the study. Because NU-GEL is not an approved treatment of DFU, future studies should compare APO-2 against standard of care.
- Placebo (culture medium processed as APOSEC(TM) diluted with 0.9% saline solution and mixed with NU-GEL) healing rates were higher in Poland and the Czech Republic than in Austria. This may have been a result of social effects. Study placebo treatment may have been more intensive as usual standard of care in these countries providing additional benefit to the patients' health and wound healing process.
- A treatment period of 4 weeks appeared to be too short to observe larger differences between APO-2 and placebo, and in future studies the treatment period should be at least 12 weeks.
- The study design was corrected during the study by expanding the eligibility criteria in protocol Version 4.0 (among other changes, patients with mild to moderate PAD were allowed to be enrolled instead of limitations to neuropathic ulcers and the wound size lower limit was reduced from 1 cm^2 to 0.8 cm^2), which accelerated recruitment and - in the APO-2 25 U/mL group - led to a higher median % reduction in wound area at 4 weeks in patients treated under protocol Version 4.0 as compared to patients treated under previous protocol versions. A Phase 3 study should be planned with the inclusion and exclusion criteria defined as in protocol Version 4.
- Patients with wounds <4 cm^2 showed a better tendency in healing.
- A central und independent assessment of the wound size for study inclusion is important and should be used in future studies.
- No differences in the results were observed between the FAS and the defined PPS.
- APO-2 was safe and well tolerated in the overall population and in patients with adjudicated wound areas ≥0.8 cm^2.

Date of report: Final 1.0 27-Nov-2024

[2] Dumville JC, O'Meara S, Deshpande S, Speak K. Hydrogel dressings for healing diabetic foot ulcers. Cochrane Database Syst Rev. 2013 Jul 12;2013(7):CD009101.