SYNOPSIS OF STUDY RESULTS

Study title: Effect of topical rhGM-CSF on the healing of venous leg ulcers: a randomized, placebo-controlled, double-blind, clinical phase II study

Protocol identifying name: Repogel-01
Clinical phase: II
EudraCT no: 2019-001483-30

Sponsor
Reponex Pharmaceuticals A/S
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Study site
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ABBREVIATIONS AND DEFINITIONS

ABI	Ankle-brachial index
AE	Adverse event
BMI	Body mass index
CFU	Colony-forming unit
GM-CSF	Granulocyte-macrophage colony-stimulating factor
IQR	Interquartile range
NPRS	Numerical pain rating scale
RCT	Randomized controlled trial
rhGM-CSF	Recombinant human granulocyte-macrophage colony-stimulating factor
SAE	Serious adverse events
SD	Standard deviation
SOP	Standard operating procedure
VLU	Venous leg ulcer

BACKGROUND

Venous leg ulcers (VLUs) are common and a substantial health care burden for society. Despite the use of evidence-based care, 25% of patients with VLUs are unlikely to have experienced healing within 6 months. Treatment with rhGM-CSF, a pluripotent cytokine/growth factor, has been reported to potentially improve wound healing outcomes in several wound types, including VLUs. In this study, the effect of topical rhGM-CSF in a hydrogel to treat difficult-to-heal VLUs was investigated.

METHODS

STUDY OBJECTIVES

The overall hypothesis of the study was that topical rhGM-CSF (molgramostim) accelerates wound healing in patients with difficult-to-heal VLUs when added to standard compression treatment and wound care. The primary objective was to study the effect of rhGM-CSF on ulcer size reduction. Further objectives were to study the effect on complete wound healing, time to complete healing, the wound healing process, safety and potential mechanisms of actions of the intervention.

STUDY DESIGN

This randomized, placebo-controlled and double-blind phase II clinical trial studied the effect of topical GM-CSF applied in a hydrogel to treat difficult-to-heal VLUs. The first patient was randomized 13-04-2021 and the last patient finished the study 19-10-2021. The total number of visits for each patient included at least 12 visits, over a trial period of a maximum of 9 weeks divided into a run-in phase of 3-5 days (D-4 to D0), a 4-week long treatment phase (D0-D28) where the study drug (Repogel) or placebo was applied twice a week, and a follow up period of 4 weeks (D28-D56), Table 1.

Table 1. Trial duration including follow-up period		
Period	**Duration**	**Treatment**
Run-in	3-5 days	Standard care
Drug/placebo	4 weeks	Standard care + rhGM-CSF/placebo
Follow-up	4 weeks	Standard care

Standard of care with e.g. compression bandages (Coban 2), debridement and neutral bandages, was given in all patients throughout the study. Participants attended study site on the following days: D-4 (screening), D0 (randomization), D3, D7, D10, D14, D17, D21, D24, D28, D38 and D56 (last visit).

CHANGES IN THE CONDUCT OF THE STUDY AND PLANNED ANALYSIS

The current study was temporarily stopped by the Danish Medicines Agency 08. Nov. 2021, following an inspection at sponsors site, due to insufficient validation of the analysis methods of the study drug in the dosage form (Repogel) in batch BA055HBMH. rhGM-CSF in the hydrogel dosage form used in the study were shown as physically not stable due to the hypothesis of stress during manufacturing process caused alteration of peptide chain over the time. As subjects were included sequentially and participating in the study over a 6-month period, the content of rhGM-CSF may have varied significantly between the patients. At this timepoint six patients were included in the study. With the necessary documentation, the study could be continued. Despite multiple attempts, it was observed that Repogel dosage form was not robust enough and quantification of rhGM-CSF was not reproducible. It was concluded that to achieve the necessary validation/documentation was not possible and therefore it was decided to stop the study and report on the six patients included. Finally a closure of the

study was filed in January 2025 with the results of six patients only and without any statistically analysis due to very small sample size (n=6).

A dose of 5 μg of rhGM-CSF (molgramostim) per cm^2 of wound bed was aimed at being used in the study. The study drug was administered twice a week for 4 consecutive weeks. The maximal dosage of molgramostim that could be given at each administration in our trial, 375 μg.

RESULTS

DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

In total 153 patients were prescreened to assess initial eligibility, of which six patients (five males and one female) were screened and subsequently included in the study. The most common reason for non-inclusion was a too small ulcer size (< $2cm^2$), low ABI (<0.7) or comorbidities. The first patient was randomized 13-04-2021 and the last patient finished the study 19-10-2021. All patients finished the study with no loss to follow-up or missing visits. The median age was 70.1 years (IQR 60.3-75.3) with a median ulcer duration of 6 months (IQR 4.0-7.7) in the placebo group (n=3) and 8 months (IQR 5.5-11.0) in the Repogel group (n=3). All patients but one (receiving Repogel) had a combination of deep and superficial venous insufficiency.

PRIMARY ENDPOINT

Proportion of patients reaching a 40% ulcer area reduction, or more, 4 weeks after initiation of the study drug treatment/placebo

The primary endpoint was reached in one of three patients in the placebo group and in none of the three patients in the Repogel group. Due to the very few numbers of enrolled patients no conclusion can be drawn because of insufficient data needed for statistical analysis.

SECONDARY ENDPOINT

Absolute and percentage change of the ulcer area 4 and 8 weeks after randomization

The mean absolute ulcer area reduction 4 weeks after randomization on D28 was 4.5 cm^2 (SD 5.3) in the placebo group (n=3) and -0.8 cm^2 (SD 1.6) in the Repogel group[1] (n=3). The mean absolute ulcer area reduction 8 weeks after randomization on D56 was 6.3 cm^2 (SD 5.3) in the placebo group (n=3) and -5.2 cm^2 (SD 8.0) in the Repogel group (n=3).

The mean percentage ulcer area reduction 4 weeks after randomization on D28 was 26.5% (SD 33.7) in the placebo group (n=3) and 1.8% (SD 10.5) in the Repogel group (n=3). The mean percentage ulcer area reduction 8 weeks after randomization on D56 was 65% (SD 20.1) in subjects receiving placebo (n=3) and -10.5% (SD 56.6) in those receiving Repogel (n=3), Fig 3. Individual ulcer area data is presented in Fig 1.

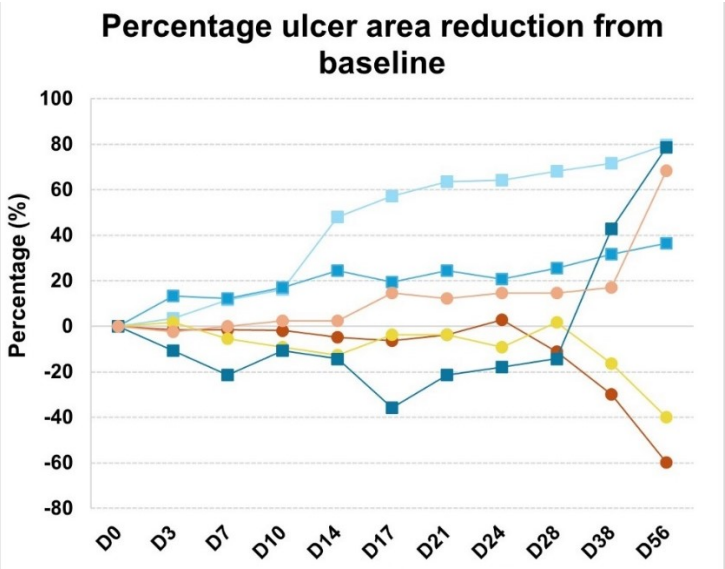

Fig 1. Individual percentage ulcer area reduction from randomization. Squares represents patients receiving placebo and circles received Repogel.

[1] Minus indicates an increase in ulcer area

One subject in the Repogel group experienced an increase in ulcer area in the follow-up period, reported as an AE and being suspected to be related to insufficient compression therapy. Therefore, standard compression (Coban 2) was switched to Profore for this patient (a 4-layer bandage; reported as a protocol deviation). The ulcer size subsequently improved very slowly, after finishing the study.

Complete ulcer healing (full epithelialization and no drainage of wound fluid) 4 and 8 weeks after randomization

There was no case of complete ulcer healing 4 or 8 weeks after randomization.

Time to complete ulcer healing

No study participant experienced complete healing during the investigation.

Degree of inflammation, granulation tissue, necrosis/slough, exudation and infection

Overall, the levels of inflammation in terms of erythema intensity were judged as mild to moderate for all patients (grading: none, mild, moderate, severe), except for one subject receiving placebo that was judged with severe inflammation D0 and D56, including one subject in the Repogel group on D14. No trend in changes during the trials was observed in either group. Exudate levels remained unchanged or improved during the study. One patient in the placebo group had an AE registered “Streptococci in wound” on D21.

Clinical improvement of the wound healing process

All patients in the placebo group experienced improvement of the wound characteristics throughout and at the end of the study. In the Repogel group, two patients were judged as unexpectedly less recovered wound at D56 for reasons possibly not related to the study drug, while one subject was

judged as having an improvement at the end of the study. Granulation tissue was normal at almost all visits for all patients or improved during the study.

Moisture meter values: The moisture meter values (expression of fluid content in the skin; the higher the value the greater the fluid content), fluctuated heavily during the study with no clear tendency in either study group.

Perilesional temperature: The maximal temperature in the ulcer and the adjacent skin were measured, with no clear trend in either study group. The highest temperature measured was always the perilesional skin, not the ulcer bed or ulcer edge itself.

Changes in the levels of cytokines and growth factors in the wound fluid

The levels of cytokines (VEGF, TNF-α, IL-1α, IL6 and GM-CSF) were assessed at baseline D0, D14 and D28, Table 2. For both study groups the levels of cytokine showed large interindividual variability at baseline D0. Cytokine levels measured at baseline (D0), Day 14 (D14), and Day 28 (D28) demonstrated that except for TNF-α which was higher in the Repogel group, all other evaluated cytokine levels group were comparatively lower in the Repogel group. Levels of GM-CSF increased in the Repogel group.

Table 2: Level of various cytokines observed during the study.

Cytokine	Group Mean (n=3)	D0	D14	D28
VEGF (pg/mg)	Placebo	635	781	1228
	Repogel	501	771	809
TNF-α (pg/mg)	Placebo	9	14	7
	Repogel	39	38	31
IL-6 (pg/mg)	Placebo	1083	1133	1077
	Repogel	1084	918	495
IL-1α (pg/mg)	Placebo	1353	426	1317
	Repogel	179	134	NA
GM-CSF (pg/mg)	Placebo	24	15	32
	Repogel	24.9	81	59

Changes in the aerobic and anaerobic microbiome

The total CFU count remained stable between D0 and D14 for all patients, apart from one patient (Repogel) that experienced a decrease in CFU. There was no obvious trend in changes in CFU of a specific bacterial species in any group. *S.aureus* was the predominant bacterial species in all patients. No patient was colonized with *P. aeruginosa*.

Assessment of the safety profile including adverse events, physical findings, biochemistry, vital signs, pain and scar evaluation

Adverse Events: No SAEs were reported during the trial. AEs were reported in four out of six patients (67%); two patients in the placebo group and in the Repogel group, respectively. A total of nine AE's developed, of which four were judged as mild, and five as moderate, Table 3. Two AE's developed in subjects receiving placebo and 7 AEs developed in the Repogel group. None but one

AE (Repogel) was judged to be possibly related to the study drug/placebo, being increasing alkaline phosphatase (increasing from 126 U/L (ref. value: 34-105) before randomization to 158 U/L, D14. Vitamin D was prescribed due to concomitant vitamin D insufficiency, and the levels of alkaline phosphatase normalized to 127 U/L on D28. One wound infection developed in the placebo group, reported D21. At both D0 and D14, this patient had growth of Streptococcus intermedius but due to no clinical suspicion of infection at these timepoints, this bacterium was initially not chosen to be treated. At D21 phenoxymethylpenicillin was prescribed for 10 days due to pain and as clinical infection could not be excluded. The pain and infection quickly resolved during the study period. One patient in the Repogel group had a significant increase in ulcer size in the follow-up period and was reported as an AE. This patient had severe venous insufficiency, and it was judged as the AE was likely related to insufficient compression therapy. The standard compression Coban 2 (a 2-layer compression system) was changed to Profore (a 4-layer compression system) due to insufficient compression for this subject. It was judged as having minor impact on data quality and patient safety. Following the end-of trials visit the patient continued treatment as prior inclusion, in the lymphoedema clinic at the study site. Slowly the ulcers improved, stopping the study.

Table 3. Adverse event displayed in the two study groups. The only adverse event judged by the blinded investigator as possibly related to the study drug /placebo is marked with a star*

Placebo		Repogel	
Adverse event (AE)	**Severity**	**Adverse event (AE)**	**Severity**
New wound (small) on index leg. Unknown cause.	Mild	Muscular lumbar pain; participant slipped at work	Mild
Wound infection (streptococci)	Moderate	Worsening of wounds on index leg (including index wound and other wounds) most likely due to insufficient compression with Coban 2	Moderate
		Bursitis olcecrani, right arm	Moderate
		Allergic exanthema (due to ichtopaste; a wound care product)	Moderate
		Worsening of hypertension	Moderate
		Increasing alkaline phosphatase*	Mild
		D-vitamin insufficiency	Mild

Vital signs: Overall, all vital signs remained stable over time except for one subject receiving Repogel with sign of hypertension during the study with an increase in systolic blood pressure from 161 mmHg D-4 to 199 mmHg on D14. This was reported as an AE with prescription of antihypertensive treatment. The systolic blood pressure subsequently improved in the study. A slight tendency of a decrease in systolic and diastolic blood pressure was noted in two subjects receiving placebo but was not judged as an AE and with no accompanying symptoms.

Pain: In general, the treatment was well-tolerated in both study groups. At baseline, prior study drug/placebo administration, patients in the placebo group tended to experience stronger wound pain with a median NPRS of 3 (range 1-8) in the placebo group and NPRS 0 (range 0-2) in the Repogel group (NPRS ranges 0 to 10, with 0 being no pain and 10 being worst imaginable pain). Two of the three patients in the placebo group required analgesic medication for pain management (paracetamol and morphine) at baseline and during the study, while none of the patients in the Repogel group required pain medication. The pain either remained stable during the study, or improved, as judged by impact on daily activities and NPRS results. Patients in the Repogel group described minor or no pain at inclusion and throughout the study (pain evaluation since last visit judged as: none, mild, moderate or severe). All patients, in both study groups, judged the intervention as being “perfectly acceptable” or “acceptable” at all treatment occasions.

Blood work-up: Overall, laboratory values of blood serology remained unchanged during the study (hemoglobin, platelet count, white blood cell count and differential, serum CRP, ALAT, basic phosphatase, bilirubin, albumin, potassium, sodium, and creatinine, and INR).

Physical signs: Each patient had a whole-body physical examination performed at D-4 and D28. No changes were described since D0, except for one subject receiving placebo who had developed a new small wound on D28, reported as an AE. The body mass index (BMI) remained stable between D-4 and D28 in both study groups.

Scar evaluation: Scar evaluation was not performed as no ulcer healed during the study period.

Conclusions

No firm conclusions about the efficacy and safety of rhGM-CSF can be drawn from this study due to insufficient data and very few number of enrolled patients (n=6). The small sample size and the insecurity about the study drug content challenges comparison between the two study groups. The placebo group showed a wound area reduction, compared to the Repogel group. However, it is challenging to assess whether this represents a true difference in effect or random variability due to the small sample size (n=3 in each treatment group). For this reason, test of statistical significance was deemed as inappropriate. No serious adverse events were reported. All patients, in both study groups, judged the intervention as being “perfectly acceptable” or “acceptable” at all treatment occasions. The trials for wound healing using rhGM-CSF are subjected to restart in future once a robust, stable and validated dosage form is developed. Therefore, sponsors are committed and engaged in developing a stable, validated and robust dosage form before recommencing trails.