1. TITLE PAGE

Marsyas I:
A Prospective Randomized Double-Blinded Placebo controlled, explorative Phase I trial to investigate the Safety and Tolerability of two different doses of Topically Administered APOSEC™ in Healthy male subjects with Artificial Dermal Wounds

Name of investigational product:	APOSEC™
Name of Sponsor:	Medical University of Vienna, Department of Surgery Spitalgasse 23, 1090 Vienna, Austria Represented by Univ.-Prof. Dr.med.univ. Michael Gnant
Protocol No.:	MarsyasI
Eudract-Number:	2013-000756-17
Development Phase of Study:	Phase I
Study Initiation date:	23.10.2014
First subject in:	27.10.2014
Last subject out:	22.05.2015
Study completion date:	11.06.2015
Investigator(s):	Univ.-Prof. Dr.med.univ.Michael Wolzt
Name of Author/Company:	Michaela Schaden, MSc Medical University of Vienna Clinical Trials Coordination Centre (KKS)
Date of report:	05.02.2016

2. SYNOPSIS

Title of Study: Marsyas I A Prospective Randomized Double-Blinded Placebo controlled, explorative Phase I trial to investigate the Safety and Tolerability of two different doses of Topically Administered APOSEC™ in Healthy male subjects with Artificial Dermal Wounds	
Investigator: Univ.-Prof. Dr.med.univ.Michael Wolzt	
Study Center: Medical University of Vienna, Department of Clinical Pharmacology	
Study Period: I	**Phase of Development:** Phase I
Name of Investigational Medicinal Product: APOSEC™	
Name of active ingredients: IL-8/CXCL8 (Interleukin-8/ CXC-Motiv-Chemokin 8) EGF (Epidermal Growth Factor) TGF-ß (Transforming Growth Factor ß)	
Primary Objective: To investigate safety and tolerability of 2 different doses of APOSEC™	
Inclusion Criteria: - Healthy male subjects with 18-50 years of age at the day of inclusion - Written informed consent will be obtained prior to screening examination - BMI of 19-27 (extremes included) - Subjects are in good clinical and mental health as established by medical history, physical examination, vital signs, electrocardiogram, results of biochemistry, hematology, virology and urine analysis at the Screening Visit **Exclusion Criteria:** - Lack of willingness or capacity to co-operate appropriately - Regular use of medications - History of malignancies - History of wound healing abnormalities - Chronic dermatological disease - History of chronic autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, diabetes mellitus, Lupus erythematodus - Tattoos in the region of planned punch biopsy - Positive HIV serology or evidence of active hepatitis - Allergy requiring medical treatment within 4 weeks before study initiation - Active infection of fever > 38°C within 7 days prior randomisation - Blood donation within 4 weeks before study initiation - Clinically relevant abnormalities in the laboratory testing, vital signs, ECG or physical examination - Participation in another clinical trial with an investigational day within 4 weeks before study participation	
Test product, dose and mode of administration Autologous lyophilized APOSEC™ formulated in Nugel for topical administration. 1ml per wound (initial dose and maintenance dose) - Group A (low dose group): derived from $25x10^6$ irradiated lyophilized PBMC and amount of lyophilized APOSEC™ will be reduced by 50% and dissolved in Nugel	

- Group B (high dose group): derived from $25x10^6$ irradiated lyophilized PBMC, containing 0 – 5214 IL-8 (pg/mL), 25 – 228 EGF (pg/mL), 2575 – 21732 TGF-ß (pg/mL) dissolved in Nugel.

Preparation: Lyophilisate was resuspended in 200µl 0.9% NaCl until complete dissolution (~30 seconds). The complete dissolved lyophilisate was mixed with 800µl NUGEL.

Duration of treatment

7 days

Reference therapy, dose and mode of administration

Placebo: Parallel induced cell-free control lyophilisate formulated in Nugel for topical administration. 1ml per wound (initial dose and maintenance dose)

Preparation: Lyophilisate was resuspended in 200µl 0.9% NaCl until complete dissolution (~30 seconds). The complete dissolved lyophilisate was mixed with 800µl NUGEL.

Criteria for Evaluation

(Modified) Intention to treat set

This analysis set includes subjects who were randomized and received at least one dose study drug.

Per-protocol set

This analysis set comprises all subjects who received study drug (at least one dose) and did not violate the protocol in a way that might affect the evaluation of the effect of the study drug(s) on the primary objective, i.e., without major protocol violations.

Statistical Methods

Primary endpoint analysis

- Assessment of Adverse Events, Serious Adverse Events and Dose-limiting Toxities

All safety and tolerability data were listed by treatment and subject for the ITT population. Subjects in the PP population were identified with an asterisk.

Secondary endpoint analysis

- Changes in wound size between baseline and End of Treatment (EOT) assessed by photographic analysis.
- Presence or absence of complete wound closure at End of Treatment (EOT) after Verum vs. Placebo administration.
- Scarring formation with respect to induration of palpable scar tissue, assessed via a caliper. Scar formation was assessed in practical conduct of the study only via photographic assessment.
- Presence or absence of re-epithelization and angiogenesis assessed by the markers CD31 and vWF.
- Investigator satisfaction assessment on applicability of the gel

All variables were presented using descriptive statistical techniques. Continuous data were summarized by treatment using the following descriptive statistics: number, mean, standard deviation (SD), minimum, median, and maximum. Categorical data were summarized by treatment as the number and percentage of treated punch biopsies. The primary variables were summarised for subjects in both the ITT and PP population. All other variables regarding the secondary endpoint will be summarised for subjects in the ITT population only.

Sample size calculation:

The outlined study was considered to be conducted as an explorative pilot study. Therefore, no statistical hypothesis was tested or rejected and the study was not powered accordingly. It was assumed by the Sponsor that 10 subjects are sufficient to detect major impacts of APOSEC™ on safety and tolerability in healthy volunteers. Subsequently no formal sample size calculation was performed.

Summary-Conclusion

Safety Results:

All events assessed as local tolerability effects or adverse events, were evenly distributed among Verum and Placebo treated wounds, in both low and high dose group. No tendency of increased occurrence of adverse events was observed in the high dose group. All adverse events were graded as not serious and mild, moreover the events documented as local tolerability effects were graded maximum up to 3 on the 5 point score. All events were graded as unexpected due to the exploratorive Phase I stage of the study. Regarding the relatedness classification, a probable relation to the IMP was determined by the Investigator only for the itching wound in proximal and distal area (subject CRF Nr.14), as well as the local tolerability effect graded 01 (faint, minimal erythema, subject CRF Nr.07 having premature terminated after test treatment). The local tolerability effects documented for subjects the first two enrolled subjects (CRF Nr.1 and 2) were not regarded as the primary reason for premature discontinuation.

After evaluation of clinical laboratory, vital signs and physical examinations at the End-of-Study Visit on day 14, no abnormalities were observed.

Efficacy Results:

No distinct difference between the low and high dose of Verum and Placebo could be detected with respect to wound healing and condition, wound and scar size assessment as well as wound closure. However re-epithelization of Verum treated wounds was initiated earlier when compared to Placebo treated wounds (day 3 for Verum treated wounds vs. day 4 Placebo treated wounds). No considerable difference was observed among the dose groups of APOSEC™ regarding the degree of re-epithelization. Neither undermining and tunnelling, nor an exsudate on the dressing were observed for any subject in both dose groups for both Verum and Placebo.

Given the rather high standard deviations no considerable difference could be detected in the photographic analysis between Verum and Placebo treated wounds for wound and scar size. At the End-of-treatment on day 7, wound closure was neither observed in any of the dose groups of APOSEC™ at the assigned location, nor in the location assigned to Placebo.

The Investigator's Evaluation of Applicability of the final formulation of the IMP was uniform with the highest grade „Extremely satisfied (5) on a scale of 5-point Likert scale.

Conclusion:

From the safety evaluation it can be concluded that the application of the Investigational Medicinal Product, both the Verum APOSEC™ and the parallel-induced cell-free medium used as Placebo control resuspended in the vehicle substance NUGEL are safe for topical administration.

Regarding the absence of a significant difference in the wound and scar geometry of Verum and Placebo treated wounds in both dose groups, the relatively small artificial wound area and differences in the baseline wound area occurring due to technical reasons in the course of the punch biopsy shall be mentioned. From a dermatological perspective, it can be concluded that the anticipated time span for detection of wound closure was defined too narrow regarding the fact the wound closure was neither present for Verum nor Placebo on day 7.

Re-epithelization of Verum treated wounds appeared to be initiated earlier when compared to Placebo treated wounds, no considerable difference was however observed among the dose groups of APOSEC™ regarding the degree of re-epithelization. The absence of undermining and tunnelling, exsudate on the dressing in both dose groups for both Verum and Placebo revealed that the IMP had no inflammatory effect on the wounds.

The uniform high Investigator's Evaluation of Applicability revealed that this formulation is very appropriate for topical administration.

Date of report: 05.02.2016