CLINICAL STUDY REPORT

According to ICH-E6 (GCP), chapter 5.22 and ICH-E3 (Study Reports)

Study Title:	INVESTIGATOR-INITIATED CLINICAL RESEARCH TRIAL ON THE EFFECTS OF 5% MINOXIDIL TOPICAL FOAM ON GENE EXPRESSION, HAIR GROWTH AND SCALP MICROENVIRONMENT IN MEN WITH ANDROGENETIC ALOPECIA
Investigational Product:	Minoxidil
Indication:	Androgenetic alopecia
Sponsor:	Charité-Universitätsmedizin Berlin Prof. Dr. med. Ulrike Blume-Peytavi, MD Department of Dermatology and Allergy Clinical Research Center for Hair and Skin Science Charité Campus Mitte Charitéplatz 1 10117 Berlin-GERMANY Tel.: + 49 30 450 518 122 Fax: + 49 30 450 512 952 E-mail: ulrike.blume-peytavi@charite.de
Protocol Code:	CRC-AGA.M-A-11
EudraCT-Number:	2013-004130-15
Study Phase:	none
Date First Patient in:	19.05.2014
Study Completion Date (Date Last Patient Out):	29.09.2014
Name/ Affiliation of Principal Investigator:	Prof. Dr. med. Ulrike Blume-Peytavi Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Dermatology, Venereology and Allergy

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GCP and Archiving Statement:	This study was conducted in accordance with local law ("Deutsches Arzneimittelgesetz" and "GCP-Verordnung"), the Declaration of Helsinki, ICH-GCP and the study protocol as presented in this report. The study documents will be archived for 15 years: according to protocol version 2.1 reflecting GCP-Verordnung (§13, paragraph 10)
Date of the Report:	17-October-2021

1. Report Synopsis of Study XXX

Name of Sponsor/ Company: Prof. Dr. med. Ulrike Blume-Peytavi Department of Dermatology, Venerology and Allergology Clinical Research Center for Hair and Skin Science Charité-Universitätsmedizin Berlin	Individual Study Table Referring to Part of the Dossier: n.a. Volume: n.a. Page: n.a.	(*For National Authority only*)
Name of Finished Products: Regaine® Männer Schaum		
Name of Active Ingredients: Minoxidil		
Title of the Study: INVESTIGATOR-INITIATED CLINICAL RESEARCH TRIAL ON THE EFFECTS OF 5% MINOXIDIL TOPICAL FOAM ON GENE EXPRESSION, HAIR GROWTH AND SCALP MICROENVIRONMENT IN MEN WITH ANDROGENETIC ALOPECIA (protocol version 2.1, date: 28.04.2014)		
Investigators: Principal Investigator: Prof. Dr. med. Ulrike Blume-Peytavi, MD Deputy Principal Investigator: Prof. Dr. med. Annika Vogt, MD		
Study center: Clinical Research Center for Hair and Skin Science Department for Dermatology and Allergy Charité-Universitätsmedizin Berlin Charité Campus Mitte Charitéplatz 1 10117 Berlin – GERMANY		
Publication (reference): No publications at the date of the report.		

Studied period (years): **Date of first enrolment:** 19.05.2014 **Date of last subject completed:** 29.09.2014	**Phase of development:** none

Objectives:

Explorative intra-individual comparison of hair growth, scalp microenvironment and RNA expression in affected (vertex) and unaffected (occiput) scalp areas of AGA subjects under the influence of minoxidil

Variables:

Global photography of the scalp

Macrophotography of the scalp

TrichoScan® (computer-aided method for the determination of hair growth and hair density activity)

Hair shaft thickness by OCT

Hair follicle size by ultrasound

RNA expression by microarrays

pH-value of scalp skin

Sebum content of scalp skin

Mini-zone CSSS for protein extraction to determine e.g. inflammatory mediators and hormone receptors

Number of patients (planned and analyzed):

Planned volunteers: 12

Analyzed volunteers: 12

Diagnosis and main criteria for inclusion:

Main criteria for inclusion:

Male subjects 21- 45 years of age

Male AGA based on a discernible hair loss in temple and vertex region rating Hamilton-Norwood Scale IIIv vertex to IV (See Appendix Hamilton-Norwood Scale)

Test product, dose and mode of administration, batch number:

Generic Name: Regaine® Männer Schaum

Formulation: Foam

Active Ingredient: Minoxidil

Amount per unit: 50 mg/g

Administration: Topical on scalp

Batch number: Not known

Packaging: aerosol can (3 x 60 ml)

Storage: storage not above 25 °C

Manufacturer McNeil Consumer Healthcare GmbH, 41431 Neuss.

Duration of Treatment:

9 weeks

Criteria for evaluation:

- Change of the parameters (variables / outcome/ methodology) between Baseline and End of study (week 9).
- Trend of the change in parameters (variables / outcome/ methodology) during the course of the study (week 5, week 9)

Security:

- Local intolerance
- AEs local and systemic
- SAEs local and systemic

Assessments were performed at baseline on week 1: V1, V2, V3; Week 5: V4, V5, V6 and week 9: V7, V8, V9

Statistical methods:

All parameters are described descriptively using means and scatter values. Inferential statistics does not apply. RNA-microarray data will be subjected to computational biostatistics analysis.

Summary – Conclusions:

Summary of Results:

Demographic data and family history of hereditary hair loss

With one exception all volunteers were Caucasian, their average age was 31.7 years with duration of AGA-related hair loss of 2 to 13 years. 66.7% report a history of hair mass reduction in the father, and 50% reported it for the grandfathers. Concerning baldness, 41.7% and 50% reported a family history for the father and the grandfathers, respectively.

Influence of minoxidil on physiological hair and scalp parameters

Among different local tolerability parameters assessed, only two subjects experienced mild (grade 1) burning at the beginning of the topical treatment. During the short-term use of topical Minoxidil in healthy volunteers, no significant systemic cardiovascular effects were observed. Blood pressure did not change, whereas Minoxidil increases heart rate slightly by 1-2 beats per minute.
Two parameters that describe the physiological condition of the scalp are sebum content and pH-value of the scalp surface. The casual sebum content was not influenced by topical Minoxidil application. The scalp sebum extraction rate showed no differences between treated and non-treated areas and it is possible to conclude that the application of the foam causes no dryness of the scalp. The natural protective acid mantle of the scalp and the hairs were measured by the pH, which had an average value of around 5.5. The measured pH values were stable over the entire period of the study and were not affected by the Minoxidil treatment.

Influence on clinical hair growth parameters:

Consistent with the expected shedding effect of minoxidil responders, 9/12 individuals experienced a reduction in the anagen/telogen ratio (A/T) at week 4 (4 in a pronounced drop with subsequent

recovery, while the others showed a trend of less pronounced but prolonged A/T reduction over 8 weeks).

RNA expression in response to minoxidil treatment:

In addition to phototrichogram assessment, 20 hair follicles were plucked from AGA-affected vertex and from lower occiput of Hamilton-Norwood IIIv-IV patients (n=12) before, 4 and 8 weeks after topical minoxidil 5% foam treatment. RNA was extracted (RNeasy Kits, Qiagen) quality checked via Agilent 2100 Bioanalyzer and submitted for hybridization (Miltenyi Biotec GmbH, Germany) using to the Agilent 60-mer Whole Genome Oligo Microarray protocol. Differential gene expression (combat method, unadjusted Anova and Tukey's Posthoc test p values) and gene set enrichment analyses (GSEA, CAMERA method, C2 collection of 4762 curated gene sets, Molecular Signatures Database) were performed. Differential gene expression analyses revealed 412 significantly down-regulated genes at week 4, while 563 were upregulated (approximately half of those remained upregulated over the whole treatment period). *GSEA yi*elded association with 188 pathways, almost all of them predominantly downregulated mostly involving transcription, translation, RNA and protein metabolism. Interestingly however, downregulated rapamycin sensitive genes were among the highly represented pathways, while this same gene set was among the pathways predominantly upregulated in vertex compared to occiput before the beginning of treatment. In comparison, changes in gene expression between week 4 and 8 weeks were less pronounced (42 additionally down- and 58 up-regulated genes). The pathways assigned to these genes were predominantly upregulated with interferon signature pathways being highly represented, but also stat3 targets and angioproliferation. Interestingly, gene expression changes in the clinically unaffected and untreated occiput area reacted in parallel, e.g., the profiles of the vertex at week 4, with 975 genes differentially expressed compared to Day 0, showed only minor differences to the corresponding occiput region.

Conclusion:

We herein applied a recently presented a clinical study protocol for RNA analysis in plucked hair follicles to identify putative markers of androgenetic alopecia to monitor gene expression in plucked hair follicles during the early phase of minoxidil treatment.

The data obtained in this set-up give deep insights in the changes, which the epithelial compartments undergo during the early phase of minoxidil treatment, but may also reveal further information on hair cycle regulation in general. The reproducible results obtained by choosing two time points (week 4, week 8) and the comparison of affected and unaffected areas prior to treatment support the validity of our findings.

I hereby confirm that the data in the results report were collected properly and are correct.

Date of the report: Month, Day, Year

Print Name:

Signature: