Effects of Wobenzym® plus in healthy, sportive people after eccentric exercise - a randomized, two-stage, double-blind, placebo-controlled cross-over trial

Synopsis

Version Final 1.0

Study Code

BTS 651/12 EudraCT: 2012-005003-40

Sponsor

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idv
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1. GENERAL STUDY INFORMATION

Effects of Wobenzym® plus in healthy, sportive people after eccentric exercise - a randomized, two-stage, double-blind, placebo-controlled cross-over trial

Title:	Effects of Wobenzym® plus in healthy, sportive people after eccentric exercise - a randomized, two-stage, double-blind, placebo-controlled cross-over trial
Name of test medication:	Wobenzym® plus.
Design:	Randomised, two stage, double-blind, placebo-controlled cross-over trial
Name of the sponsor:	MUCOS Pharma GmbH & Co. KG
Protocol:	Protocol BTS651/12 – EudraCT 2012-005003-40, Version Final 1.4, April 8, 2013
Phase of development:	IV
Study Center(s):	1 centre
Name of sponsor signatory:	Stefanie Rau, MUCOS Pharma GmbH & Co. KG
Date of SAP Stage I:	January 20th, 2014
Date of SAP Stage II:	December 17th, 2014

2. SYNOPSIS

Name of company: Mucos Pharma GmbH & Co. KG	**Summary table referring to part of the dossier**	**(For National Authority use only)**
Name of finished product: Wobenzym® plus	**Volume:** **Page:**	
Name of active ingredient: 450 F.I.P.-U bromelain, 24 µkat trypsin and 100 mg rutoside (rutoside · 3 H_2O)		

Title:	Effects of Wobenzym® plus in healthy, sportive people after eccentric exercise - a randomized, two-stage, double-blind, placebo-controlled cross-over trial
Principal investigators:	[redacted]
Study centers:	[redacted]
Study Duration:	Stage I: 35 days Stage II: 7 days
Clinical phase:	IV
Publications:	None
Objectives:	Multidimensional approach for acute phase and recovery after eccentric stress test based on reduction of maximal concentric strength and pressure induced pain (final SAP Stage II, version 3.0, 17.12.2014).
Methodology:	Monocenter, double-blind, randomized, placebo-controlled, crossover study (stage I) designed to assess acute phase and recovery after eccentric stress test. Stage II: parallel group design.

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Number of patients planned and analyzed:	In the presence of a two-stage crossover design with a medium-sized period-correlation of 0.5 the total required sample size for the primary multidimensional hypothesis resulted in 2 x 20 subjects. With this total sample size of 40 subjects, a „medium-sized" group difference (MW = 0.64) with regard to the multivariate outcome ensemble could be detected with a power of 90%. The completion of stage I was defined as recruitment of about 2/3 of the planned study subjects. If neither success nor futility was formally determined after stage I, a stage II study was to be planned anew based on the results of stage I (sample-size re- assessment with adaptive design features according to Bauer and Köhne). Due to normal variations (drop outs) the planned sample size was increased to 28 for stage I. Stage I Randomized 30 (15 subjects in each sequence, A-B and B-A) Safety Set 28 (15 subjects A-B, 13 subjects B-A) Full Analysis (ITT) Set 27 (15 subjects A-B, 12 subjects B-A) Per-Protocol (PP) Set 26 (14 subjects A-B, 12 subjects B-A) The recalculated total sample size for stage II was 2 x 22 patients (sample size re-assessment according to protocol and final stage I SAP (version 1.0, 20.01.2015), one phase, no crossover design in stage II). Stage II Randomized 44 (22 Wobenzym® plus, 22 Placebo) Safety Set 44 (22 Wobenzym® plus, 22 Placebo) Full Analysis (ITT) Set 43 (22 Wobenzym® plus, 21 Placebo) Per-Protocol (PP) Set 41 (20 Wobenzym® plus, 21 Placebo)
Diagnosis and main criteria for inclusion:	For inclusion in the study, prospective volunteers had to meet all of the following inclusion criteria: • Subject is in good physical and mental health as established by medical history, physical examination, electrocardiogram, vital signs, results of biochemistry, haematology • Not anticipating any planned changes in lifestyle regarding activity and nutrition for the duration of the study • Non smoker • Men with strength training experience

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	• Age: 20-50 years • BMI ≥20 kg/m^2 and ≤ 32 kg/m^2 • medium concentric strength ability (150-300 Nm) • Subject is able and willing to sign the Informed Consent Form prior to screening evaluations
Dosage and administration:	The medications used in this study were: 1. Investigational product • Name: Wobenzym® plus • Dosage: 4 tablets, three times a day • Route of administration: oral 2. Placebo • Dosage: 4 tablets, three times a day • Route of administration: oral. The subjects in the placebo group received tablets of similar size and colour containing no active ingredients. They were given identical instructions for consumption.
Test product	Wobenyzm® plus One enteric-coated tablet Wobenzym® plus contains 450 F.I.P.-U bromelain, 24 µkat trypsin and 100 mg rutoside (rutoside · 3 H_2O). Excipients: tablet core: lactose monohydrate, corn starch, stearic acid 70, fine-particle silicium dioxide, talcum, magnesium stearate, purified water; tablet coat: Eudragit® L12,5 (methacrylic acid methyl methacrylate copolymer (1:1), talcum, macrogol 6000, triethyl citrate, vanillin, titan dioxide, 2-propanol, purified water.
Reference therapy	Placebo No active ingredients. Active ingredients were substituted by microcrystalline cellulose. Excipients: tablet core: lactose monohydrate, stearic acid 70, fine-particle silicium dioxide, talcum, magnesium stearate, purified water; tablet coat: Eudragit® L12,5 (methacrylic acid methyl methacrylate copolymer (1:1), talcum, macrogol 6000, triethyl citrate, vanillin, titan dioxide, 2-propanol, purified water.
Duration of treatment:	Intake of study preparation started 3 days before the eccentric exercise and lasted until 3 days after the eccentric exercise at each part of the study.

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Criteria for evaluation:	**Primary endpoint** (Final SAP Stage II, version 3.0, 17.12.2014)**:** Reduction of maximal isokinetic strength after stress test Pressure induced pain: Nm/cm measured with algometry (in the middle of the muscle belly, m. rectus femoris) (Multidimensional approach for acute phase and recovery after eccentric stress test based on reduction of maximal concentric strength and on pressure induced pain)	
Statistical methods:	Minimizing the required assumptions is a recommended approach for confirmatory statements on efficacy. This applies especially in scales with skewed distributions including floor and ceiling effects as is known from many scales used for evaluation of recovery after eccentric exercise. Thus, a non-parametric assessment of treatment effects independent of data type and distribution was chosen as the primary analysis method. The analysis was performed within the framework of a two-phase-crossover-analysis using the Wei-Lachin procedure, a multivariate generalization of the Wilcoxon-Mann-Whitney test, which takes account of the correlation among univariate Mann-Whitney tests for each outcome to produce an overall average estimate of benefit and test for treatment differences. The multiple level alpha of the study (global level of significance for the whole study) was defined as alpha = 0.025, one-sided test for superiority. According to the ICH Guideline E9 the results were given as P-values as well as effect size measures with their confidence intervals (Mann-Whitney statistic as corresponding effects size measure of the Wilcoxon-Mann-Whitney test), so that the direction and quantity of the treatment effects are determined with their precision. The Mann-Whitney effects size measure (MW) gives the probability that a randomly chosen subject of the test group is better off than a randomly chosen subject of the comparison group, defined in statistical shortcut: P (X<Y) + 0.5 P (X = Y).	

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Applying the Mann-Whitney effects size measure, the null and alternative hypothesis for the comparisons of the test treatment to control treatment (superiority) can be formulated as follows:

H_0: $MW_{TC} \leq 0.50$
H_A: $MW_{TC} > 0.50$

H_0: Null-hypothesis; H_A: Alternative Hypothesis; T: Test Treatment; C: Control

The traditional benchmarks for the Mann-Whitney effects size measure (MW) are as follows:

0.29	large inferiority
0.36	medium inferiority
0.44	small inferiority
0.50	equality
0.56	small superiority
0.64	medium superiority
0.71	large superiority

The sequence and nature of the a priori ordered multidimensional hypotheses of the study was as follows (final SAP):

1. Multidimensional Outcome Ensemble Acute Phase (2 criteria, 2 points in time)
   - Reduction of maximal isokinetic strength after stress test at 3h, 6h (multivariate analysis)
   - Pressure induced pain: Nm/cm measured with algometry (in the middle of the muscle belly, m. rectus femoris) at 3h, 6h (multivariate analysis)

2. Multidimensional Outcome Ensemble Recovery Phase (2 criteria, 2 points in time)
   - Reduction of maximal isokinetic strength after stress test at 24h, 48h (multivariate analysis)
   - Pressure induced pain: Nm/cm measured with algometry (in the middle of the muscle belly, m. rectus femoris) at 24h, 48h (multivariate analysis)

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If the first multidimensional test shows statistical significance, the second multidimensional hypothesis could then be tested in a confirmatory manner according to the principle of a priori ordered hypotheses (with the same alpha as the first multidimensional approach). The procedure of a priori ordered hypotheses is most powerful with full control of the multiple level of alpha.

The two-stage adaptive procedure of Bauer and Köhne was chosen as the sequential method. Stage I decision was to be performed with the following decision structure (global multiple level alpha = 0.025 one-sided, p_1 = P-value of stage I,p_2 = P-value of stage II)

a)	$p_1 \geq \alpha_0 = 0.5$:	stop because of futility
b)	$p_1 \in (0.0102; 0.5)$:	continue with stage II
c)	$p_1 \leq \alpha_1 = 0.0102$:	stop with success (rejection of H_0)

The predefined decision structure was to be applied to the first confirmatory hypothesis. For stage I decision according to (c) the sample size was to be recalculated according to Bauer-Köhne based on p_1 and $\alpha_c = 0.0038$ (one-sided).

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Summary and Conclusions:
Efficacy Results:

The study was originally planned as a two-phase crossover-trial within the framework of a two-stage procedure according to Bauer-Köhne.

After stage I, the crossover approach had to be abandoned due to statistically significant inequality of carry-over effects. This situation prevented the originally planned evaluation as a crossover trial and the study was continued in stage II following a classic parallel group design.

The multivariate test of the first confirmatory hypothesis of stage I (multidimensional ensemble of peak torque and pressure induced pain at 3h and 6h) resulted in P = 0.0332 (one-sided, directional test for superiority, Wei-Lachin procedure). The associated effect size MW (Mann-Whitney) indicated a more than "small" superiority of the test group (MW = 0.6153). The P-value of the first primary hypothesis was lying within the decision boundaries for stage II:

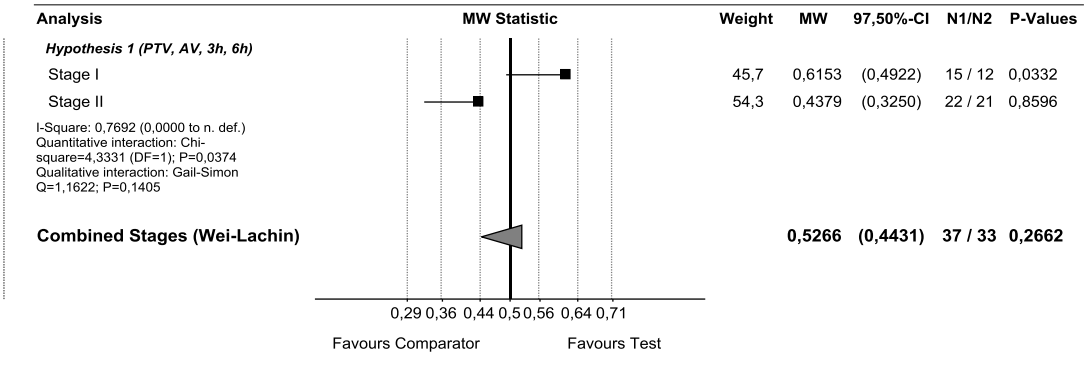
$$\alpha 1 \leq 0.0332 < 0.5$$

Thus, in accordance with the SAP and due to the unequal carryover effects, the trial was continued with stage II based on a parallel group approach (stage II sample size: $N_{Stage\ II} = 40$).

For stage II, the multivariate test of the first confirmatory hypothesis resulted in P = 0.8596 (one-sided, Wei-Lachin procedure), thus providing no evidence for corresponding treatment effects in the stage II population. This result is in contrast to the result found for the stage I population (see above).

Figure 1 shows the result of the formal meta-analysis of the two stages for hypotheses no. 1 with the corresponding test for heterogeneity (I-Square values above 0.5 indicating "large" heterogeneity).

Figure 1



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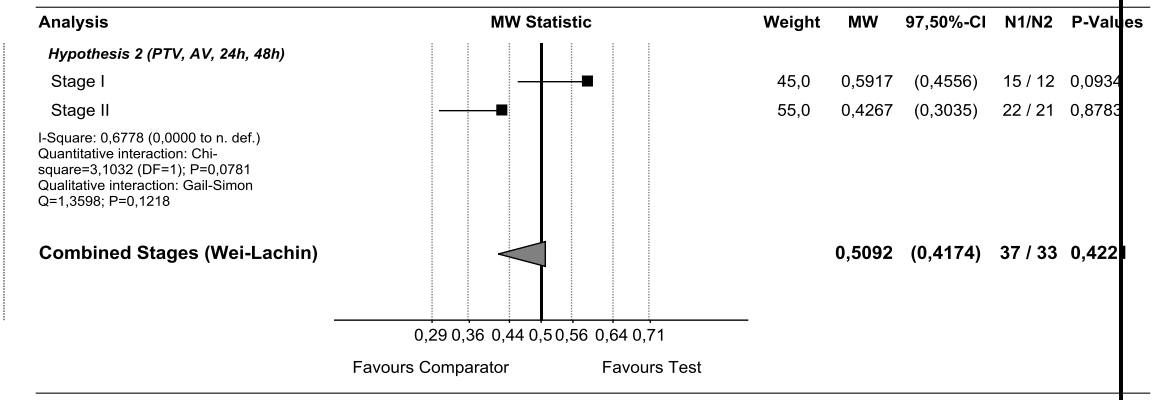
As shown in the above figure, there is some indication for "severe" heterogeneity of the two stages (I-Square = 0.7692, P = 0.0374). Thus, the common precondition for a formal combination of the two stages was not fulfilled and, as defined in the final stage II SAP for such a case, the results regarding hypothesis no. 1 are to be interpreted separately for each stage.

The next figure shows the formal meta-analysis for hypotheses no. 2 (multidimensional ensemble of peak torque and pressure induced pain at 24h and 48h):

Figure 2



Again, opposite results are found for the two stages (MW = 0.5917 vs. MW = 0.4267) with "severe" heterogeneity (I-Square = 0.6778, P = 0.0781). Thus, the results of hypothesis no. 2 should be interpreted separately for each stage.

How can the observed marked stage differences be explained? Already during the final blind review analyses, i.e. after completion of stage II, severe differences were found for the pooled treatment groups regarding the baseline status of the two stages, e.g.:

- pressure induced pain at baseline, stage I vs stage II, P< 0.0001
- "healthy" status according to NK-cell-test: 14% in stage I population vs. 60% in stage II population

One explanation might be that in the first stage predominantly less trained endurance sportsmen were enrolled, while in stage II well-trained fitness sportsmen and athletes in team sports with higher and specialized activity levels were enrolled (also demonstrated by the markedly higher baseline peak torques). This way, in stage II treatment effects might have been masked by ceiling effects so that no additional benefit could be detected for the stage II volunteers.

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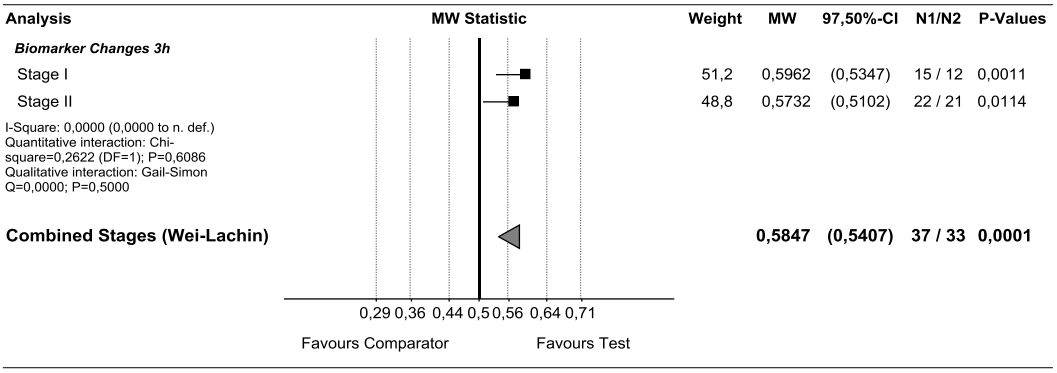
With respect to biomarkers a multivariate analysis was performed for the changes at 3h (the point in time where all biomarkers were measured). The pooled effect size shows a "more than small" superiority of the test group (MW = 0.5847, P = 0.0002, Wei-Lachin procedure). Both stages are also stand alone statistically significant with respect to these secondary criteria. It is notable that there was no indication for heterogeneity of the stages (I-Square = 0.0), as opposed to the situation for peak torque and pain measurements (see above). Also applying the Bauer-Köhne pooling of the two stages (as originally planned for the confirmatory hypotheses), the overall result for the biomarkers is statistically significant (p_1p_2 0.000013 < α_C 0.0038; one-sided).

The following figure shows the multivariate result of the biomarkers for the single stages and for the combined stages:

Figure 3



Safety Results:

In stage I, 13 adverse events were reported in 10 subjects during the individual phase with Wobenzym® plus treatment and 3 adverse events were reported in 3 subjects during the individual phase with Placebo treatment (subject P106 with adverse events in both treatment phases). No adverse event was "serious". Two events were assessed as "severe" (P109, severe headache, relation "not related", day 4 of phase 2, phase treatment: Wobenzym® plus; P118, severe effusion, relation "not related", day 6 of phase 1, phase treatment: placebo).

Out of 16 single adverse events, 13 events were assessed as "not related", two as "unlikely", and one event as "possible" (P102, mild diarrhea, relation "possible", day 1 of phase 2, phase treatment: Wobenzym® plus).

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In stage II, two adverse events were reported in one subject of the "Wobenzym® plus" group (P220) and in no patient in the placebo group. Both events were "mild" and "resolved". One event was assessed as "not related", and one event as "possible" (P220, mild acne-like rush at chin and mild pruritus, relation "possible", Wobenzym® plus).

Regarding blood routine parameters there no clinically relevant findings in any of the treatment groups.

Conclusions:

Peak torque measurements and algometry showed some effects in stage I with less trained endurance sportsmen. These results could not be reproduced in stage II with the well-trained fitness sportsmen and athletes in team sports. The stage differences were statistically significant and the I-Square measure indicated marked heterogeneity. Thus, the results of the two stages have to be interpreted separately.

Further investigation is needed for in-depth explanation of the observed stage heterogeneity.

Regarding biomarkers there was good indication for homogeneity of the two stages (I-Square = 0) and the combined result at 3h after baseline (the point in time where all biomarkers were measured) showed significant superiority of the Wobenzym® plus group for each stage as well as for the combined stages (changes from baseline, MW = 0.5847, P = 0.0001).

All results are to be interpreted in an exploratory manner.

Date of Report: 13 July 2015 (Version Final 1.0)