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4 Article type : Article

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7 **TITLE PAGE**8 **Title:**

9 First-in-human trial assessing the pharmacokinetic-pharmacodynamic profile of a novel  
10 recombinant human chorionic gonadotropin in healthy women and men of reproductive  
11 age.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/CTS.13037](#)

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5 **Conflicts of Interest:**

6 All authors are present employees of Ferring Pharmaceutics A/S and there are no other  
7 relationship or activities that could appear to have influenced the submitted work

8 **Funding:**

9 This trial was funded by Ferring Pharmaceuticals A/S, Copenhagen, Denmark

10 **Keywords:**

11 Recombinant hCG, choriogonadotropin beta, FE 999302, human cell line,  
12 pharmacokinetics, pharmacodynamics, testosterone

13 Presented at the 35<sup>th</sup> Annual Meeting of the European Society of Human Reproduction  
14 and Embryology, 23-26 June 2019, Vienna, Austria

## Abstract

The purpose of this first-in-human trial was to examine the safety, pharmacokinetics and pharmacodynamics of a novel recombinant human chorionic gonadotropin (FE 999302, choriogonadotropin beta) to support its clinical development for various therapeutic indications. The single and multiple dose pharmacokinetics of choriogonadotropin beta (CG beta) were evaluated in women and the single dose pharmacokinetics and pharmacodynamics of CG beta were compared to those of choriogonadotropin alfa (CG alfa) in men. CG beta was safe and well-tolerated in all 84 healthy subjects. In women, the area under the curve (AUC) and the peak serum concentration ( $C_{\max}$ ) increased approximately dose proportionally following single and multiple doses of CG beta. The apparent clearance (CL/F) was approximately 0.5 L/h, the mean terminal half-life approximately 45 hours and the apparent distribution volume ( $V_z/F$ ) approximately 30 L. After single administration in men, the mean AUC was 1.5-fold greater for CG beta than for CG alfa. Mean  $C_{\max}$  and  $V_z/F$  were comparable for the two preparations. In accordance with the differences in AUC, the CL/F was lower for CG beta (CL/F 0.5 vs 0.8 L/h), explained by a longer terminal half-life (47 vs 32 hours). Serum testosterone levels induced by a single dose rhCG reflected the pharmacokinetic profiles with a slight delay, resulting in 59% higher AUC for CG beta. The pharmacokinetic parameters for CG beta were comparable in men and in women. In conclusion, the pharmacokinetics differs between the two rhCG preparations, causing higher exposure and a higher pharmacodynamic response for CG beta, which may require relatively lower therapeutic doses.

## Introduction

Human chorionic gonadotropin (hCG) is a glycoprotein hormone that is produced by the pituitary in small amounts in non-pregnant women, men and menopausal women whereas large amounts are produced by the placenta of pregnant women.<sup>1, 2</sup> During early pregnancy, hCG is first expressed by the blastocyst before implantation and is increasingly produced after implantation by the syncytiotrophoblast. During the first trimester of pregnancy, hCG is produced in increasing amounts up to 10<sup>th</sup> week of

1 gestation and then decreases gradually.<sup>3</sup> Since intact hCG is cleared by the kidneys,  
2 hCG may be isolated from the urine of women and used for the manufacturing of  
3 therapeutic preparations.<sup>4, 5</sup>

4 HCG consists of a 92 amino acid single  $\alpha$ -subunit, which is common to all the pituitary  
5 glycoprotein hormones, and a specific  $\beta$ -subunit of 145 amino acids. Each subunit is post  
6 translationally modified by the addition of complex carbohydrate moieties. The alpha  
7 subunit contains 2-N-linked glycosylation sites at amino acids 52 and 78 and the beta  
8 subunit contains 2-N-linked glycosylation sites at amino acids 13 and 30 and four O-  
9 linked glycosylation sites at amino acids 121, 127, 132 and 138.<sup>6, 7</sup> HCG and luteinizing  
10 hormone (LH) shows similar molecular structures and interact with the same LH/CG  
11 receptor.<sup>8</sup> As a result of this similarity to LH, hCG is used pharmacologically in a number  
12 of clinical indications. In women, hCG is used to induce final follicular maturation  
13 following controlled ovarian stimulation or to induce ovulation in anovulatory women.<sup>9</sup> In  
14 men with hypogonadotropic hypogonadism, hCG is given to induce and maintain  
15 spermatogenesis.

16 To date, there is only one approved recombinant hCG (rhCG) preparation  
17 (choriogonadotropin alfa, CG alfa) which is expressed by a Chinese Hamster Ovary  
18 (CHO) cell-line and the pharmacokinetics are similar to those of urinary hCG.<sup>10, 11</sup>

19 Choriogonadotropin beta (CG beta, FE 999302) is a novel rhCG that has been produced  
20 by a human cell line (PER.C6<sup>®</sup>). The amino acid sequence of the  $\alpha$ - and  $\beta$ -chains of CG  
21 beta are identical to those of endogenous hCG and CG alfa. Glycosylation of both natural  
22 and recombinant hCG is highly complex and may contain a wide range of structures.<sup>12</sup>

23 The glycosylation of recombinant hCG reflects the range of glycosyl-transferases present  
24 in the host cell line and is known to differ between rhCG products produced by different  
25 cell lines.<sup>13</sup>

26 PER.C6<sup>®</sup> and CHO cell lines are both used for production of recombinant follicle-  
27 stimulating hormone (rFSH), with follitropin alfa expressed by a CHO cell line and  
28 follitropin delta expressed by the PER.C6<sup>®</sup> cell line. Investigations show that the  
29 preparations of rFSH from the PER.C6<sup>®</sup> human cell line and a CHO cell line display  
30 important differences in pharmacokinetic and pharmacodynamic properties.<sup>14</sup> These  
31 differences include consistently higher exposure, longer time to  $C_{max}$ , and longer  $t_{1/2}$  of  
32 follitropin delta after a single administration, and longer  $t_{1/2}$  at steady state after repeated



administrations, compared with follitropin alfa. A significantly lower clearance of follitropin delta compared with that of follitropin alfa was as well measured. Based on these differences which can be attributed to the glycosylation profile it may be anticipated that the pharmacokinetic and pharmacodynamic properties of rhCG expressed by a human cell line and by a CHO cell line will also be dissimilar.

To examine the safety, pharmacokinetics and pharmacodynamics of CG beta, the first-in-human trial comprised three parts conducted sequentially and included healthy women using oral contraceptives and healthy men down-regulated with GnRH agonist. The single and multiple dose pharmacokinetics of CG beta were first evaluated in healthy women and then the single dose pharmacokinetics and pharmacodynamics were compared to those of CG alfa in healthy men. The goal of this research was to establish the pharmacokinetics and pharmacodynamics of CG beta in women and men over a broad dose-range in order to allow further development of CG beta for any potential therapeutic indication.

## Methods

### Participants

This first-in-human trial of CG beta included 84 women and men. Eligible participants were women 18-40 years of age or men 18-50 years of age with a body mass index (BMI) of 18-29 kg/m<sup>2</sup>. All participants were healthy according to medical history, physical examination (including gynaecological examination in women), a 12-lead ECG, and clinical laboratory profiles of blood and urine. Written informed consent was obtained from all subjects prior to inclusion in the trial which was conducted in accordance with the Declaration of Helsinki and International Council for Harmonization–Good Clinical Practice. The trial was approved by the Ethical Committee (EC) of the Bavarian Chamber of Physicians, Germany.

### Study design

This trial was composed of three parts including only women in parts 1 and 2 and men in part 3. All women were required to have used combined oral contraceptive (ethinylestradiol content  $\geq 0.015$  mg) or combined contraceptive vaginal ring for at least 3 cycles prior to trial inclusion. All women were switched to Yasmin® (Bayer) contraceptive

1 tablets 14 days prior to CG beta administration and this contraceptive was taken daily  
2 throughout the study period. Men were down-regulated with a depot GnRH agonist  
3 (triptorelin, Decapeptyl®, Ferring Pharmaceuticals) to suppress endogenous hormone  
4 production.

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## 7 **Part 1**

8 The first part of the trial had a double-blind, placebo-controlled and randomised single  
9 ascending dose design and included 35 women. Divided in 5 cohorts (5 active treatment,  
10 2 placebo in each cohort), 25 women were dosed with a single dose of CG beta and 10  
11 women were dosed with placebo. The dose levels of CG beta were 4, 16, 64, 128, and  
12 256 µg. All doses were administered as single subcutaneous injections in the abdomen.  
13 Blood samples for measurement of serum hCG concentrations were obtained  
14 immediately before administration of CG beta or placebo and at 2, 5, 8, 10, 12, 14, 16,  
15 24, 36, 48, 72, 96, 120, 144, 168, 216 and 264 hours after administration.

## 16 **Part 2**

17 The second part of the trial had a double-blind, placebo-controlled and randomised  
18 multiple ascending dose design and included 16 women. Divided into 2 cohorts (6 active  
19 treatment, 2 placebo in each cohort), 12 women were dosed daily with CG beta and 4  
20 women were dosed with placebo. The daily CG beta dose levels were 8 and 16 µg  
21 administered as single subcutaneous injections in the abdomen for 10 consecutive days.  
22 Blood samples for measurement of serum hCG concentrations were obtained  
23 immediately before administration of each CG beta or placebo doses, and then at 2, 5, 8,  
24 10, 12, 14, 16, 24, 36, 48, 72, 96, 120, 144, 168, 216 and 264 hours after the last dose.

## 25 **Part 3**

26 The last part of the trial had an open randomised 2-way crossover design comparing the  
27 pharmacokinetics and the testosterone release after administration of CG beta and CG  
28 alfa (Ovitrelle®, Merck Serono) in 33 down-regulated men. All men received three doses  
29 of 3.75 mg Decapeptyl in order to downregulate the pituitary-gonadal axis and suppress  
30 testosterone to  $\leq 1$  ng/mL and LH to  $\leq 2.5$  IU/mL at the time of rhCG administration. Two  
31 doses of Decapeptyl were administered prior to the first drug administration on Days -28  
32 and Day -10 and a third dose was given on Day 12 after the first dose of rhCG. A single

dose of 125 µg rhCG of each preparation was administered subcutaneous in a cross-over design with the two treatment periods approximately 3 weeks apart. Blood samples for measurement of serum hCG concentrations were obtained immediately before drug administration, and then at 4, 8, 12, 14, 16, 24, 36, 48, 72, 96, 120, 168, 216 and 264 hours after each administration. Blood samples for measurement of serum testosterone concentrations were collected immediately before drug administration and then 4, 8, 12, 16, 24, 48, 72, 96, 120, 168, 216 and 264 hours after each administration.

### **Safety and tolerability**

Safety and tolerability were assessed by monitoring of adverse events (AEs), injection site reactions, clinical laboratory assessments (clinical chemistry, hematology, urinalysis), physical examination, vital signs (blood pressure, pulse, body temperature), 12-lead ECG and – in women – transvaginal ultrasonography. The summarized AEs are those reported during the treatment phase, i.e. from administration of rhCG until the last assessment 11 days after the last dose.

### **Bioanalytical methods**

Serum rhCG levels were measured using a sandwich immunoassay, comprising a monoclonal mouse anti-hCG Beta 2 as capture antibody and a ruthenium-labelled monoclonal mouse anti-hCG Holo C3 as detection antibody with electrochemiluminescence (ECL) detection (Meso Scale Discovery system). The analytical standard used was CG beta for quantification of CG beta, and CG alfa for quantification of CG alfa, and the analytical range was 0.100 – 12.0 ng/ml serum (up to 240 ng/mL with extended dilution). Inter-assay precision was ≤9%, and intra-assay precision was ≤6%, in the main method validation, as calculated using ANOVA. In order to ensure equivalent exposure CG alfa was quantitated by amino acid analysis independent of the label values.

Analysis of serum concentrations of testosterone was performed by means of a validated LC-MS/MS method.

The analyses for antibodies against rhCG were performed using a validated bioanalytical method. The method validations were designed to follow the principles stated in Shankar et al. and the regulatory guideline-15, 16 The method was a semi-homogenous bridging

1 assay using ECL as detection method. The trial samples were analysed using a tiered  
2 approach. All samples (study samples and controls) were analysed as duplicates and the  
3 mean signal was used for determination of results.

4

## 5 **Statistical analyses**

6 The pharmacokinetics, pharmacodynamics and safety were summarised using  
7 descriptive statistics.

8 Women receiving a single dose of 4 µg CG beta had serum hCG concentrations below  
9 the LOQ in 4 out of the 5 women. Therefore, it was not possible to calculate any  
10 meaningful PK variables for this dose group. In contrast, all serum hCG concentrations  
11 from other women and all men were included in the PK calculations.

12 The pharmacokinetic and pharmacodynamic parameters were calculated by non-  
13 compartmental analysis (NCA) using the software Phoenix WinNonlin® (Pharsight  
14 Corporation, USA). The pharmacodynamic parameters were calculated for baseline  
15 corrected data, assuming a constant background testosterone concentration after down-  
16 regulation. The relation between body weight (BW) and exposure was investigating for  
17 AUC and  $C_{max}$  by fitting the function  $k/BW^c$  to data using linear regression after log-  
18 transformation. Dose adjusted exposure data from all subjects in all three parts were  
19 used for this investigation. If the exponent c is different from 0 then the exposure is  
20 related to body weight. If  $c=1$  the exposure is inversely proportional to body weight.

21 Analysis of dose proportionality for AUC and  $C_{max}$  was based on the single dose groups  
22 16 to 256 µg CG beta. The slope (beta) was estimated from the model  $\log(\text{parameter}) =$   
23  $\ln(\alpha) + \text{beta} \cdot \ln(\text{dose})$ . A slope of 1 corresponds to dose proportionality.

24 Comparison of PK and PD parameters between CG beta and CG alfa in part 3 of the trial  
25 were performed using analysis of variance on log-transformed parameters, including  
26 factors for drug, period, and subject. Estimated ratios and 90% confidence intervals were  
27 derived from the model and back-transformation of log-transformed differences. The  
28 statistical analyses were performed using the software SAS.

29

## 30 **Results**

1 Thirty-five healthy women aged between 18 and 40 years were randomized and dosed  
2 for the single dose pharmacokinetic investigation (Part 1 of the trial), 7 in each treatment  
3 group. The mean body weight was 65.9 kg with a range from 50.7 to 90.6 kg, and mean  
4 BMI was 23.9 kg/m<sup>2</sup> with a range from 19.1 to 28.9 kg/m<sup>2</sup>. Overall, the treatment groups  
5 were similar with respect to demographic parameters.

6 For the repeated dose pharmacokinetic investigation (Part 2 of the trial), 16 women  
7 between 19 and 40 years of age were randomized and dosed, 8 in each treatment group.  
8 The mean body weight was 64.7 kg with a range from 55.0 to 81.4 kg, and mean BMI  
9 was 23.4 kg/m<sup>2</sup> with a range from 19.7 to 27.2 kg/m<sup>2</sup>. Overall, the treatment groups were  
10 similar with respect to demographic and baseline characteristics.

11 Thirty-three healthy men between 18 and 50 years of age were included in the single  
12 dose pharmacokinetic and pharmacodynamic investigations (Part 3 of the trial). The  
13 mean body weight was 82.6 kg with a range from 59.3 to 96.0 kg, and mean BMI was  
14 25.3 kg/m<sup>2</sup> with a range from 19.9 to 29.0 kg/m<sup>2</sup>.

#### 16 **Part 1: Single Dose PK in women**

17 The mean serum concentrations of CG beta after single dosing are shown in **Figure 1**.  
18 After administration of CG beta, serum concentrations increased until reaching the  
19 maximal concentration at 24 hours (median) with a range of 2 to 48 hours. The geometric  
20 mean maximum concentrations ( $C_{max}$ ) ranged from 0.3 to 7.7 ng/mL after single doses of  
21 16, 64, 128 and 256 µg CG beta. Subsequently the concentrations declined with a  
22 geometric mean terminal half-life across the 4 evaluable doses of 45 hours (CV%: 18%).  
23 The concentrations were approximately back to baseline level 11 days after the  
24 administration of CG beta. The geometric mean values of apparent total clearance and  
25 apparent volume of distribution were estimated to 0.48 L/h (CV%: 30%) and 31 L (CV%:  
26 31%), respectively, across the evaluable doses.

27 The AUC and  $C_{max}$  were approximately dose proportional within the analysed dose range  
28 16-256 µg. The slope (Beta) was estimated to 1.14 (95% CI 1.00-1.28) for AUC and 1.20  
29 (95% CI 1.01-1.38) for  $C_{max}$ .

#### 31 **Part 2: Multiple Dose. PK in women**

32 The mean serum concentrations of CG beta after single dosing are shown in **Figure 1**.

Following the daily administration of CG beta over 10 days, the trough concentration increased and reached steady-state after 6-7 days in the 8 µg group and after 7-8 days in the 16 µg group. The median time for reaching maximal serum CG beta concentrations after the last CG beta dose was 10 hours (range 5-16 hours) after multiple dosing. The geometric mean  $C_{max}$  values were 0.69 ng/mL (CV%: 32%) in the 8 µg dose group and 1.9 ng/mL (CV%: 21%) in the 16 µg dose group. Two subjects in the 8 µg group showed substantially lower exposure compared to the rest of the subjects in this dose cohort. The geometric mean terminal half-life across the 2 doses was 42 hours (CV%: 15). The geometric mean values for the apparent total clearance and apparent volume of distribution of CG beta were 0.45 L/h (CV%: 40%) and 27 L (CV%: 50%), respectively.

### Part 3 Single Dose PK in men

The mean serum concentrations of CG beta and CG alfa after single dosing are shown in **Figure 1**.

The average time taken for the mean hCG concentration to reach  $C_{max}$  after a single injection of 125 µg CG beta compared to a single injection of 125 µg CG alfa was around 24 hours for both compounds. The geometric mean serum  $C_{max}$  were also comparable being 2.59 ng/mL (CV%: 40%) after CG beta administration and 2.59 ng/mL (CV%: 73%) after CG alfa administration. However, in spite of similar  $C_{max}$  values, exposure as determined by  $AUC_t$  was substantially different with the geometric mean  $AUC_t$  for CG beta being 50% (90% CI: 1.36-1.65) greater compared to that for CG alfa. This difference was also reflected in the geometric mean half-life which was 47 hours after a single injection of 125 µg CG beta and 32 hours after a single injection of 125 µg CG alfa and the geometric mean apparent total clearance which was 0.50 L/h (CV%: 31%) after CG beta administration and 0.75 L/h (CV%: 42%) after CG alfa administration. The geometric mean apparent distribution volumes were 34 L (CV%: 37%) and 35 L (CV%: 46%), respectively, after CG beta and CG alfa administration.

### Comparison of PK results after a single dose to women and men

Mean serum CG beta concentrations after single subcutaneous injection of 128 µg CG beta to women and 125 µg CG beta to men are shown in **Figure 2**.

After single dose administration of 128 µg CG beta to 5 women and 125 µg CG beta to 33 men the PK profiles and PK parameters for CG beta were comparable.

#### Relationship between body weight and CG beta exposure

The association between body weight and exposure in women and men is shown in **Figure 2**. Regardless of gender, both AUC and  $C_{max}$  decreased with increasing body weight. The power exponent for body weight was 0.85 (95% CI [0.36;1.35],  $p=0.0009$ ) for AUC and 1.12 (95% CI [0.61;1.63],  $p<.0001$ ) for  $C_{max}$ , indicating that both AUC and  $C_{max}$  declined approximately proportionally to the inverse of the body weight.

#### Part 3 Single Dose PD in men

The mean baseline corrected serum testosterone concentrations after single dosing of CG beta and CG alfa are shown in **Figure 3**.

The median time for reaching baseline corrected maximal testosterone concentration was 96 hours (range 48-168 hours) after a single subcutaneous injection of 125 µg CG beta and 72 hours (range 48-120 hours) after a single subcutaneous injection of 125 µg CG alfa with geometric mean testosterone plasma  $C_{max}$  concentrations of 7.1 ng/mL (CV%: 30%) and 6.7 ng/mL (CV%: 32%), respectively. In accordance with the concentration profiles and exposure of CG beta and CG alfa, the testosterone  $AUC_t$  was 1.6-fold (90% CI: 1.50-1.68) greater after administration with CG beta than with CG alfa.

A summary of the estimated PK parameters of CG beta in women and the estimated PK and PD parameters of CG beta and CG alfa in men is shown in **Table 1** and **Table 2**, respectively.

#### Safety

CG beta was well tolerated in both women and men after single or multiple subcutaneous injections. No severe or serious adverse events (AEs) occurred, no AE lead to discontinuation of the trial and none of the subjects developed antibodies against CG beta.

1 In Part 1, 21 AEs were reported by 12 women (48%) on active treatment and 7 AEs were  
2 reported by 5 women (50%) on placebo. There were no apparent dose-related trends in  
3 AE frequency. In Part 2, there were 12 AEs in 6 women (100%) in the 8 µg group, 35 AEs  
4 in 5 women (83%) in the 16 µg group and 12 AEs in 4 women (100%) in the placebo  
5 group. The most frequently reported AEs in women on active treatment were nausea,  
6 headache and uterine spotting. In Part 3, the frequency of AEs in down-regulated men  
7 was comparable in the two treatments, i.e. 22 AEs occurred in 15 men (45%) after CG  
8 beta treatment, and 28 AEs occurred in 18 men (55%) after CG alfa treatment, without  
9 any apparent difference between the two groups. The most frequently reported AEs in  
10 men were hot flush and headache. An overview of AEs reasonably possibly related to  
11 treatment is provided in the supplementary Tables S1-S3.

12 In Part 3, two down-regulated men experienced transient increases in ALT and AST in  
13 the second treatment period; in one subject the liver enzyme increases occurred after  
14 administration of CG alfa, in the other subject they occurred prior to and after  
15 administration of CG beta. There were no other clinically significant findings or apparent  
16 dose-related trends in physical examination, vital signs, ECG, transvaginal ultrasounds,  
17 or safety laboratory data after either single or repeated administrations in women and  
18 men.



## 1 Discussion

2 The three part design of this first-in-human trial of CG beta provides information on the  
3 safety of CG beta in healthy subjects, single and multiple dose pharmacokinetics in  
4 women, comparative single dose pharmacokinetics in men and allows a comparison of  
5 the pharmacokinetics of CG beta between genders.

6 Single ascending doses up to 256 µg were safe and well-tolerated in women and the  
7 increases in serum CG beta levels were approximately proportional with dose. CG beta  
8 serum hCG concentrations were too low to calculate meaningful PK parameters after a  
9 single dose of 4 µg, but in the other dose groups from 16 µg to 256 µg the AUC and  $C_{max}$   
10 of CG beta increased in an approximately dose proportional manner. The  
11 pharmacokinetic parameters  $t_{1/2}$ , CL/F, and  $V_z/F$  were all similar across the dose range.

12 The half-life of CG beta was longer (45 vs 29 h), the apparent clearance (0.5 vs 0.7 L/h)  
13 was lower and the apparent volume of distribution (31 vs 29 L) was comparable, when  
14 compared to available literature data of CG alfa.<sup>10, 11</sup> The difference in elimination rate  
15 between CG beta and CG alfa may be explained by the higher degree of sialylation of  
16 CG beta molecule including mono-, di-, tri- as well as tetra-sialylation structures.<sup>17</sup>

17 Following multiple daily dosing of CG beta in women, serum hCG levels accumulated and  
18 reached approximate steady state levels after 6-8 days. The estimates of  $t_{1/2}$ , CL/F, and  
19  $V_z/F$  for CG beta after daily repeated administration were similar to the estimates  
20 obtained after a single dose of CG beta (42 vs 45 h, 0.45 vs 0.48 L/h and 27 vs 31 L  
21 respectively). The median  $t_{max}$  was naturally shorter after multiple administration (8-11 h)  
22 compared to single dose administration (16-24 h) as concentrations remaining from  
23 previous doses were declining exponentially. Thus, the shift in  $t_{max}$  was mainly caused by  
24 the slow elimination of CG beta in combination with the relatively short dosing interval of  
25 24 hours.

26 In part 3 of the trial, the rhCG dose administered to down-regulated men was 125 µg for  
27 both preparations. This choice of dose was based on previous experience with urinary  
28 hCG and published data for CG alfa.<sup>10, 18</sup> A dose of 125 µg rhCG is high enough to give  
29 reliable comparative PK data and also induces sufficient testosterone production for  
30 comparative analysis (125 µg of CG alfa is approximately equivalent to 2500 IU as  
31 determined in the rat bioassay).<sup>18</sup> Administration of 125 µg CG beta and CG alfa to men  
32 resulted in considerably higher exposure (1.5 fold) to CG beta compared with CG alfa. In

line with this the estimated apparent clearance was lower and the half-life longer for CG beta when compared to CG alfa but the apparent volume of distribution after administration was similar between compounds. Despite the difference in exposure the  $C_{max}$  for serum hCG concentration increased to similar levels indicating that the absorption rate is very similar, while the elimination is slower for CG beta as supported by the lower CL/F and in accord with the higher exposure. The pharmacokinetic data of CG alfa in part 3 are in good agreement with those previously published for CG alfa.<sup>8, 14</sup> After single dose administration of 125 µg rhCG to men, the production of testosterone was higher (1.6 fold) following CG beta injection than after CG alfa injection. Maximum serum testosterone production was reached at 3 days after injection for both compounds but thereafter serum testosterone declined at a slower rate in the CG beta group than in the CG alfa group. Since the half-life of endogenous testosterone is relatively short, the slower testosterone decline reflects the longer half-life of CG beta.<sup>19, 20, 21</sup> Thus, the higher exposure and lower apparent clearance of CG beta when compared to CG alfa, resulted in sustained higher testosterone levels after CG beta administration.

The association between body weight and exposure in both women and men indicated that regardless of gender, both AUC and  $C_{max}$  decreased with increasing body weight. Other studies have shown similar associations for urinary and other recombinant hCG preparations.<sup>22, 23, 24</sup>

Comparing the pharmacokinetic properties of CG beta after a single subcutaneous administration of 125 µg in men and 128 µg in women revealed very similar pharmacokinetic profiles without any apparent gender specific characteristics. The slightly higher exposure in women is ascribed to their lower body weight rather than to the marginally higher dose. The pharmacokinetic parameters were similar regardless of gender. The differences between rhCG expressed in a human cell line and in a CHO cell line were assessed in men only. However, since the PK differences of gonadotropins between men and women are known to be limited it is to be expected that the differences between CG beta and CG alfa observed in men can also be expected in women.<sup>25</sup>

The safety profile of CG beta in this trial was reassuring with rather few AEs, all of which were of mild or moderate intensity. The most frequent reported AE in women was headache, and in men was hot flush, the latter most likely related to their testosterone

deficient status. Overall, the drug was well-tolerated and its potential immunogenicity seems low and in line with that reported for rFSH produced by the same cell line.<sup>26</sup> In conclusion, CG beta has shown to be safe and well-tolerated both in women and men. The pharmacokinetic-pharmacodynamic profile of CG beta is different from CG alfa, and since the amino acid sequences are identical, it can be inferred that the glycosylation differences are responsible for the lower clearance of CG beta in comparison to CG alfa. Due to pharmacokinetic and pharmacodynamic differences, the potential therapeutic dose of CG beta is likely to be lower, in both women and men, than that for other hCG preparations.

## Study Highlights

### What is the current knowledge on the topic?

Recombinant hCG is indicated for the treatment of male or female infertility and administered by single or multiple subcutaneous injections.

### What question did this study address?

A new recombinant hCG (choriogonadotropin beta) produced by a human-derived cell line (PER.C6) is currently in clinical development. The amino acid sequence of the  $\alpha$ - and  $\beta$ -chains are identical to the natural sequences and also to that of rhCG expressed by CHO cell line (choriogonadotropin alfa), but the glycosylation provided by the PER.C6 and CHO cells is different. In this trial, the pharmacokinetics of choriogonadotropin beta were assessed in women and men and the pharmacokinetics and pharmacodynamics were compared in men to those of choriogonadotropin alfa.

### What does this study add to our knowledge?

It is concluded that the pharmacokinetics of the two rhCG preparations are different, due to a slower clearance of choriogonadotropin beta resulting in a higher pharmacodynamic response.

### How might this change clinical pharmacology or translational science?

Further development of choriogonadotropin beta may require lower doses of this potent hCG compared to current therapeutic hCG preparations.

## Acknowledgement

The authors would like to thank Dr. Manuela Koch, who was the Principle Investigator, and the medical staff at Nuvisan GmbH as well as all volunteers who participated in the trial.

## Author Contributions

L-E.B.K., C.H., K.B. and B.M. wrote the manuscript; L-E.B.K., C.H., P.L. and B.M. designed the research; P.L. and L-E.B.K. performed the research; L-E.B.K., P.L. and K.B. analyzed the data.

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## Figure legends and tables

**Figure 1.** Time course of mean serum concentrations after single and multiple subcutaneous administrations of CG beta and CG alfa to women and men in parts 1 to 3 of the trial.

Individual serum concentrations are shown with dots and the arithmetic mean with solid lines. Standard deviation is shown with shaded areas.

The upper plot to the left shows the serum concentrations after single administration of CG beta to women in part 1 of the trial.

The upper plot to the right shows the serum concentrations after multiple administration of CG beta to women for 10 days in part 2 of the trial.

The lower plot to the left shows the serum concentrations after single administration of CG beta and CG alfa to men in part 3 of the trial.

The lower plot to the right shows the serum concentrations on a logarithmic scale after single administration of CG beta and CG alfa to men in part 3 of the trial.

**Figure 2.** Exposure of CG beta in women and men.

Upper plot: Time course of mean serum concentrations after single subcutaneous administration of 128 µg CG beta to women and 125 µg CG beta to men in part 1 and part 3 of the trial. Individual serum concentrations are shown with dots and the arithmetic mean with solid lines. Standard deviation is shown with shaded areas.

Lower plots: Body weight influence on exposure by means of AUC and  $C_{\max}$ . AUC and  $C_{\max}$  values are dose normalised to 125 µg CG beta. Solid line represents fitted regression curve.  $AUC = 10335/BW^{0.85}$  ( $p=0.0009$ ) and  $C_{\max} = 349/BW^{1.12}$  ( $p<.0001$ ).

**Figure 3.** Time course of baseline corrected serum testosterone concentrations after single subcutaneous administration of CG beta and CG alfa to men in part 3 of the trial.

Individual serum concentrations are shown with dots and the arithmetic mean with solid lines. Standard deviation is shown with shaded areas.

**Table 1.** Summary of PK parameters for CG beta after single and multiple subcutaneous administrations to women in parts 1 and 2 of the trial.

**Table 2.** Summary of PK and PD parameters for CG beta and CG alfa after single subcutaneous administrations to men in part 3 of the trial.

### **Supplementary Files**

1. Supplemental Material Table S1
2. Supplemental Material Table S2
3. Supplemental Material Table S3



**Table 1 Summary of PK parameters for CG beta after single and multiple administration to women in part 1 and part 2 of the trial**

<b>PK parameters:</b>	<b>CG beta</b>	<b>CG beta</b>	<b>CG beta</b>	<b>CG beta</b>
Single dose of CG beta administration to women	<b>16 µg</b> <b>N=5</b>	<b>64 µg</b> <b>N=5</b>	<b>128 µg</b> <b>N=5</b>	<b>256 µg</b> <b>N=5</b>
<b>AUC<sub>inf</sub> (h*ng/mL)<sup>a</sup></b>	27.8 (22)	144 (40)	288 (41)	563 (10)
<b>AUC<sub>t</sub> (h*ng/mL)<sup>a</sup></b>	18.4 (32)	132 (42)	275 (38)	553 (10)
<b>C<sub>max</sub> (ng/mL)<sup>a</sup></b>	0.305 (28)	1.37 (51)	2.94 (18)	7.74 (47)
<b>t<sub>max</sub> (h)<sup>b</sup></b>	24 [14; 36]	24 [14; 48]	24 [16; 36]	16 [2; 24]
<b>t<sub>1/2</sub> (h)<sup>a</sup></b>	46.0 (16)	47.0 (11)	46.8 (28)	42.0 (13)
<b>CL/F (L/h)<sup>a</sup></b>	0.576 (17)	0.445 (50)	0.445 (31)	0.455 (10)
<b>Vz/F (L)<sup>a</sup></b>	38.3 (27)	30.2 (48)	30.0 (18)	27.6 (12)
<b>PK parameters:</b>	<b>CG beta</b>	<b>CG beta</b>		
Multiple dose of CG beta administration for 10 days to women	<b>8 µg</b> <b>N=6</b>	<b>16 µg</b> <b>N=6</b>		
<b>AUC<sub>τ</sub> (h*ng/mL)<sup>a</sup></b>	15.0 (35)	41.9 (22)		
<b>C<sub>max</sub> (ng/mL)<sup>a</sup></b>	0.694 (32)	1.90 (21)		
<b>t<sub>max</sub> (h)<sup>b</sup></b>	8 [5; 12]	11 [10; 16]		
<b>t<sub>1/2</sub> (h)<sup>a</sup></b>	43.5 (16)	40.8 (14)		
<b>CL/F (L/h)<sup>a</sup></b>	0.534 (40)	0.382 (26)		
<b>Vz/F (L)<sup>a</sup></b>	33.5 (51)	22.5 (21)		

<sup>a</sup>= Geometric mean (coefficient of variation%), <sup>b</sup>=Median [range], N=number of subjects

AUC<sub>inf</sub>=Area under the concentration-time curve from time zero to infinity

AUC<sub>t</sub>=Area under the concentration-time curve from time zero to time of last measurable concentration (above the lower limit of quantification)

AUC<sub>τ</sub>= Area under the concentration-time curve during a dosing interval at steady state

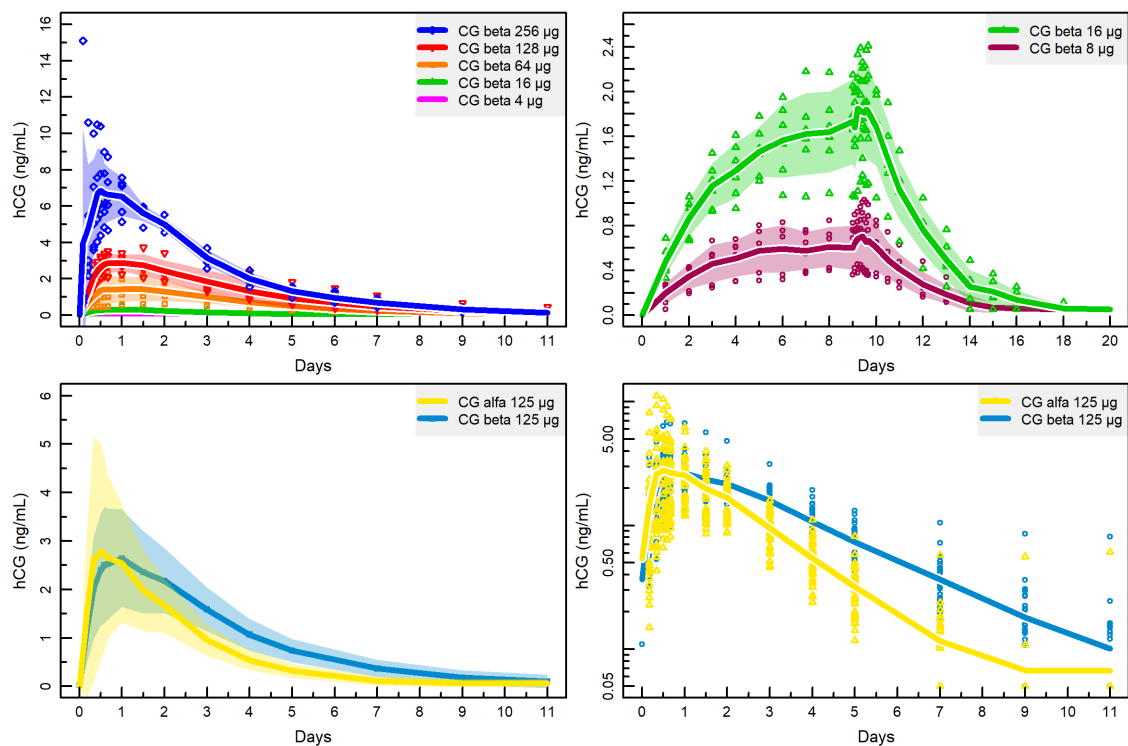
**Table 2 Summary of PK and PD parameters for CG beta and CG alfa after single administration to men in part 3 of the trial**

<b>PK parameters:</b>	<b>CG beta, 125 µg</b>	<b>CG alfa, 125 µg</b>	<b>Ratio</b>		
Single dose of CG beta and CG alfa administration to men	<b>N=33</b>	<b>N=33</b>	<b>CG beta / CG alfa</b>		
			<b>Estimate</b>	<b>90% CI</b>	<b>p-value</b>
<b>AUC<sub>inf</sub> (h*ng/mL)<sup>a</sup></b>	249 (35)	166 (45)	1.50	1.36; 1.65	p<0.00001
<b>AUC<sub>t</sub> (h*ng/mL)<sup>a</sup></b>	235 (32)	156 (46)	1.51	1.36; 1.67	p<0.00001
<b>C<sub>max</sub> (ng/mL)<sup>a</sup></b>	2.59 (40)	2.59 (73)	1.00	0.87; 1.14	0.9961
<b>t<sub>max</sub> (h)<sup>b</sup></b>	24 [12; 48]	24 [8; 48]			
<b>t<sub>1/2</sub> (h)<sup>a</sup></b>	47.1 (36)	32.3 (44)	1.46	1.41; 1.51	p<0.00001
<b>CL/F (L/h)<sup>a</sup></b>	0.503 (31)	0.754 (42)	0.67	0.61; 0.73	p<0.00001
<b>V<sub>z</sub>/F (L)<sup>a</sup></b>	34.1 (37)	35.2 (46)	0.97	0.87; 1.08	0.6501
<b>PD parameters:</b>	<b>CG beta, 125 µg</b>	<b>CG alfa, 125 µg</b>			
Single dose of CG beta and CG alfa administration to men	<b>N=33</b>	<b>N=33</b>			
	<b>Testosterone baseline corrected</b>	<b>Testosterone baseline corrected</b>			
<b>AUC<sub>inf</sub> (h*ng/mL)<sup>a</sup></b>	1219 (40)	766 (35)	1.59	1.50;1.68	p<0.00001
<b>C<sub>max</sub> (ng/mL)<sup>a</sup></b>	7.09 (30)	6.74 (32)	1.05	0.99; 1.11	0.1401
<b>t<sub>max</sub> (h)<sup>b</sup></b>	95.9 [48; 168]	72 [48; 120]			

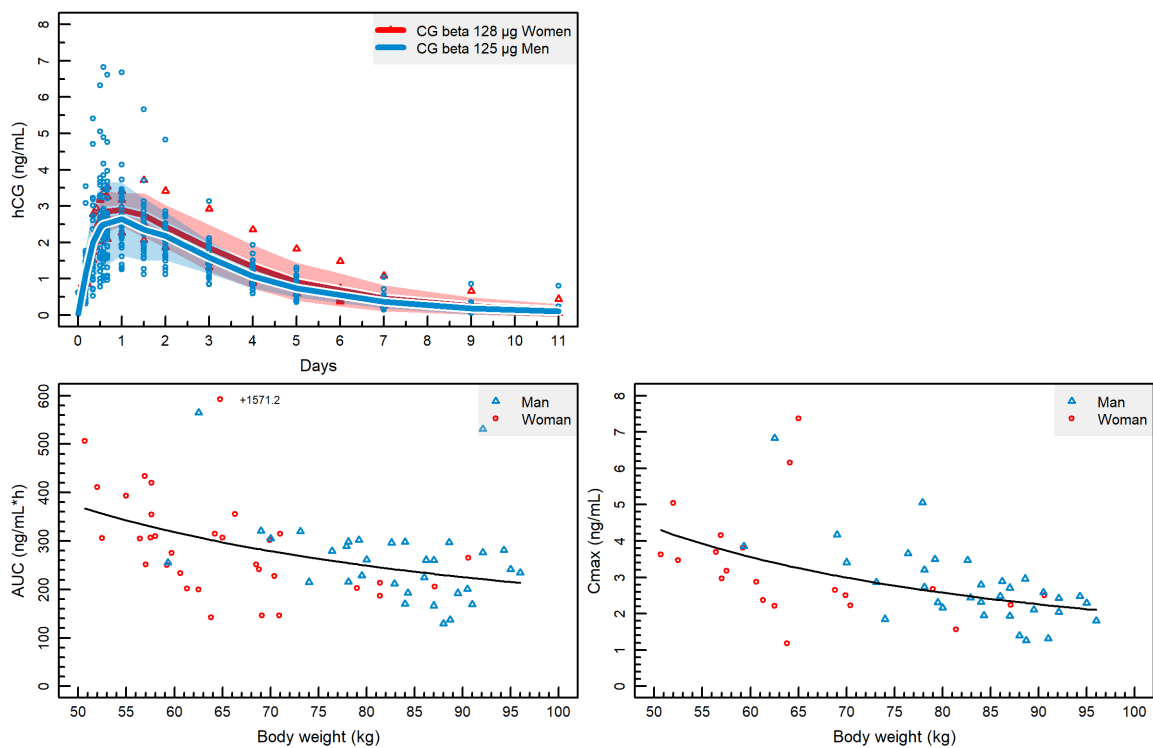
<sup>a</sup>= Geometric mean (coefficient of variation%), <sup>b</sup>=Median [range], N=number of subjects

AUC<sub>inf</sub>=Area under the concentration-time curve from time zero to infinity

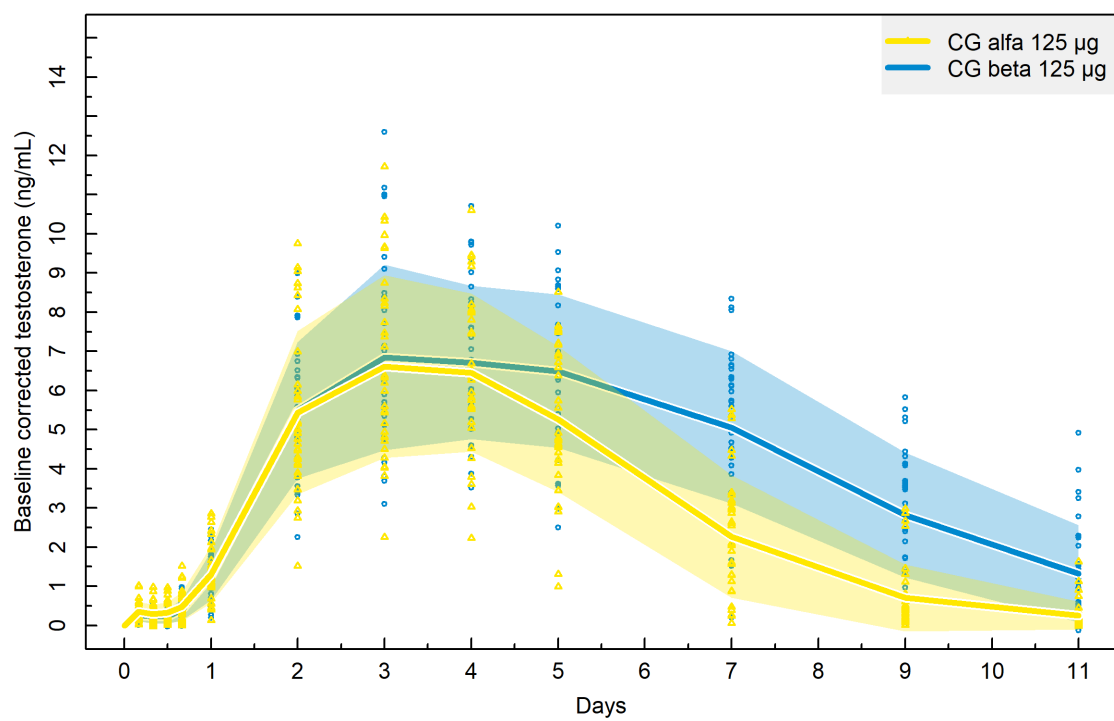
AUC<sub>t</sub>=Area under the concentration-time curve from time zero to time of last measurable concentration (above the lower limit of quantification)



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