The Journal of Clinical Endocrinology & Metabolism, 2022, **107**, 2036–2046
https://doi.org/10.1210/clinem/dgac145
Advance access publication 11 March 2022
Clinical Research Article

Corifollitropin Alfa Combined With Human Chorionic Gonadotropin in Adolescent Boys With Hypogonadotropic Hypogonadism

R. Ravi Shankar,[1] Suneri Shah,[1] Hee-Koung Joeng,[1] Geraldine Mendizabal,[1] Julia R. DiBello,[1] Yanfen Guan,[1] Barbara J. Stegmann,[2] Eberhard Nieschlag,[3] Hermann M. Behre,[4] Ronald S. Swerdloff,[5] Michelle C. Fox,[1] and Keith D. Kaufman[1]

[1]Merck & Co Inc, Kenilworth, New Jersey 07033, USA
[2]Organon & Co Inc, Jersey City, New Jersey 07302, USA
[3]Centre for Reproductive Medicine and Andrology, University Hospital Münster, 48149 Münster, Germany
[4]Centre for Reproductive Medicine and Andrology, University Hospital Halle, 06120 Halle, Germany
[5]Lundquist Research Institute, David Geffen School of Medicine at UCLA, Torrance, California 90502, USA

Correspondence: R. Ravi Shankar, MD, Merck & Co, Inc, 126 E Lincoln Ave, Rahway, NJ 07065, USA. Email: ravi_shankar3@merck.com.

Abstract

Context: Adolescent males with hypogonadotropic hypogonadism (HH) have traditionally been treated with exogenous testosterone (T) or human chorionic gonadotropin (hCG) to produce virilization; however, those modalities do not result in growth of the testes and may promote premature maturation and terminal differentiation of Sertoli cells prior to their proliferation, which may impact future fertility. Another option is to use gonadotropins in those individuals to induce testicular growth, proliferation and maturation of Sertoli cells, and production of endogenous T with consequent virilization.

Objective: We examined the efficacy and safety of corifollitropin alfa (CFA) combined with hCG for the induction of testicular growth and pubertal development in adolescent boys with HH.

Methods: This was a 64-week, multicenter, open-label, single-group study of CFA in adolescent boys, aged 14 to younger than 18 years, with HH. Seventeen participants initiated a 12-week priming period with CFA (100 μg if weight ≤ 60 kg, or 150 μg if weight > 60 kg) given subcutaneously once every 2 weeks, after which they entered a 52-week combined treatment period with CFA, once every 2 weeks, and subcutaneous hCG, twice-weekly (hCG dose adjusted between 500 IU and 5000 IU to keep total T and estradiol levels within protocol-specified ranges). The primary efficacy end point was change from baseline in testicular volume (TV), measured as the sum of volumes of left and right testes by ultrasound.

Results: After 64 weeks of therapy with CFA/CFA combined with hCG, geometric mean fold increase from baseline in TV was 9.43 (95% CI, 7.44-11.97) (arithmetic mean of change from baseline at week 64, 13.0 mL). Hormonal, Tanner stage, and growth velocity changes were consistent with initiation and progression of puberty. Treatment was generally well tolerated. No participant developed anti-CFA antibodies.

Conclusion: Treatment of adolescent boys with HH with CFA alone for 12 weeks followed by CFA combined with hCG for 52 weeks induced testicular growth accompanied by pubertal progression, increased T, and a pubertal growth spurt (EudraCT: 2015-001878-18).

Key Words: corifollitropin alfa, hypogonadotropic hypogonadism, adolescent boys, testes

Abbreviations: ADA, antidrug antibody; AMH, antimüllerian hormone; ASaT, all subjects as treated; CFA, corifollitropin alfa; CV, coefficient of variation; E2, estradiol; FAS, full analysis set; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; HH, hypogonadotropic hypogonadism; LH, luteinizing hormone; QoL, quality of life; recFSH, recombinant follicle-stimulating hormone; SHBG, sex hormone–binding globulin; T, testosterone; TV, testicular volume.

Male hypogonadotropic hypogonadism (HH) is characterized by impaired secretion of the pituitary gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH), resulting in insufficient testicular function including deficiencies in testosterone (T) production and spermatogenesis (1). HH can be the result of a primary defect in gonadotropin secretion by the pituitary gonadotrophs, or it can result from defects in gonadotropin-releasing hormone (GnRH) secretion by the hypothalamus. The clinical manifestations of HH depend on the stage of development at which the deficiency occurred (prepubertal or postpubertal). When the condition occurs before puberty (prepubertal), puberty is delayed or absent. The condition can be congenital or acquired and can occur in isolation, in association with anosmia/hyposmia, or it can occur as part of a multiple pituitary hormone deficiency syndrome (2, 3). HH in young boys is associated with significant psychosocial impact and physical consequences leading to depression, anxiety, sexual dysfunction, and a lower quality of life (QoL), all of which may improve considerably with treatment (4). The standard treatment for HH in males is androgen (T) therapy in adolescence and human chorionic gonadotropin (hCG) with or without FSH when fertility is desired.

An important goal in the treatment of adolescent boys with HH is to increase serum T levels, leading to a pubertal growth

Received: 24 October 2021. **Editorial Decision:** 8 March 2022. **Corrected and Typeset:** 16 April 2022

spurt and the development of male secondary sexual characteristics (5-7). This can be achieved either through the administration of exogenous T, or by using hCG to stimulate LH receptors on Leydig cells of the testes, thereby inducing the production of endogenous T. Another important goal in the treatment of adolescent boys with HH is to stimulate spermatogenesis, at an appropriate time, to support future fertility. Treatment with hCG alone can induce spermatogenesis, especially in patients with testicular volume (TV) greater than 4 mL at baseline (8), but may require prolonged treatment (9, 10). In contrast, a shorter duration of treatment with gonadotropins (both FSH and hCG) to induce puberty during adolescence has been reported to result in spermatogenesis (11). While the sequence of gonadotropins used in these treatment regimens to induce puberty has varied, when FSH is initiated before stimulation of the LH receptor, increases in TV, serum inhibin B levels into the adult range, and Sertoli cell and spermatogonia numbers in the testes, as well as a reduction in Sertoli cell to germ cell ratio (by biopsy) have been reported (3) when compared to a group in which both FSH and LH receptors were stimulated simultaneously from the start of therapy. Historically, adolescent boys with HH were not treated with FSH, as support of fertility was not the main goal of treatment at this age (5-7). Instead, these patients were administered exogenous T in a sufficient quantity to induce the development of male secondary sexual characteristics, and from then on, to maintain normal male T levels. However, T therapy alone does not induce testicular changes necessary to support fertility. It is possible that adolescent boys who are not exposed to FSH during the critical pubertal window required for Sertoli cell proliferation may miss this crucial period in testicular development; however, data on the responses to fertility induction in men with HH treated with T during adolescence are scant. Meta-analyses or reviews of the literature suggest mixed results, and it is possible that this results from a combination of patients with prepubertal as well as postpubertal onsets of HH (12, 13). One study that assessed this reported mixed responses to fertility-inducing therapies in men who were treated with T in adolescence (14). Treatment with hCG alone to induce endogenous production of T has the potential of inducing terminal differentiation of Sertoli cells before they have had the opportunity to undergo proliferation and maturation (3).

Treatment protocols using gonadotropins to induce puberty in adolescent boys with HH have involved pretreatment with hCG, followed by combined treatment with hCG and FSH or pulsatile GnRH (11, 15); or simultaneous initiation of hCG and FSH or use of pulsatile GnRH (16); or pretreatment with FSH followed by combined treatment with hCG and FSH or pulsatile GnRH (2, 17). Stimulation of endogenous T production with hCG is preferred over administration of exogenous T to induce virilization because hCG induces higher levels of intratesticular T, which promotes spermatogenesis and can stimulate additional testicular growth if the testes are still at a suboptimal volume (6). Inhibin B has been shown to be a useful surrogate for monitoring spermatogenic activity in boys when semen analysis is not feasible (18).

Owing to its short half-life, recombinant FSH (recFSH) needs to be injected multiple times per week to maintain therapeutically effective levels; a treatment for HH that requires fewer injections may be more acceptable to adolescent boys and may result in fewer medication errors and improved adherence. Corifollitropin alfa (CFA; Elonva) is a recombinant gonadotropin consisting of the α-subunit of human FSH and a hybrid subunit composed of the sequence of the β-subunit of human FSH and the C-terminal peptide part of the β-subunit of hCG. CFA acts at the same gonadal FSH receptor as recFSH, but it has an approximately 2-fold longer elimination half-life and an almost 4-fold increase in time to peak serum levels (T_{max}) compared with recFSH. A single injection of CFA replaces 7 days of daily recFSH injections when used to induce the development of ovarian follicles in women undergoing controlled ovarian stimulation during assisted reproductive technologies (19). For the treatment of males with HH, a single injection of CFA every 2 weeks is intended to replace FSH injections administered 2 to 3 times per week. A previous study in men with HH demonstrated that CFA administered once every 2 weeks in combination with hCG for 52 weeks increased TV significantly and induced spermatogenesis in 77% of patients who had remained azoospermic after a 12-week treatment with hCG alone (20). The present study examined the efficacy and safety of 64 weeks of therapy with CFA administered once every 2 weeks (alone for 12 weeks and then combined with hCG for weeks 12-64) in adolescent boys aged 14 to younger than 18 years with HH. The sequence of gonadotropin therapy used in this study, with CFA alone (priming period) followed by combined treatment with CFA and hCG, was chosen to approximate normal pubertal changes as well as to optimize increases in Sertoli cell number (and TV) before their maturation due to exposure to T.

Materials and Methods

Patient Selection Criteria

Adolescent boys who met the following key criteria were eligible to participate in the study: age 14 to younger than 18 years; established diagnosis of HH (either congenital or acquired with onset before puberty); TV less than 4.0 mL for each testicle, as determined by ultrasound; and circulating levels of total T less than the lower limit of normal of 8.3 nmol/L, FSH less than or equal to 2 IU/L, LH less than or equal to 2 IU/L, and inhibin B levels less than or equal to 35 ng/L.

Boys were excluded from the study if any of the following key criteria were met: history of bilateral cryptorchidism (maldescended testes) or unilateral cryptorchidism treated after age 2 years; history or presence of clinically significant testicular problems (eg, epididymitis, orchitis, testicular torsion, varicocele grade III, testicular atrophy, occlusive azoospermia) that in the opinion of the investigator would impair the individual's response to treatment; had known damage or injury to the vas deferens; previous treatment with GnRH, gonadotropins (eg, hCG, FSH), or androgens (eg, T); untreated or inadequately treated pituitary or hypothalamic tumor; uncontrolled endocrinopathies, including thyroid, adrenal, and pituitary disorders not on stable replacement therapies (ie, individual had not been on stable doses for at least 3 months); history of active pituitary hypersecretion as evidenced by hyperprolactinemia or Cushing disease, or acromegaly, or any other active pituitary hypersecretion syndrome; hypophysectomy within a period of 12 months before the start of screening; allergy/sensitivity to gonadotropins or its/their excipients.

Study Design

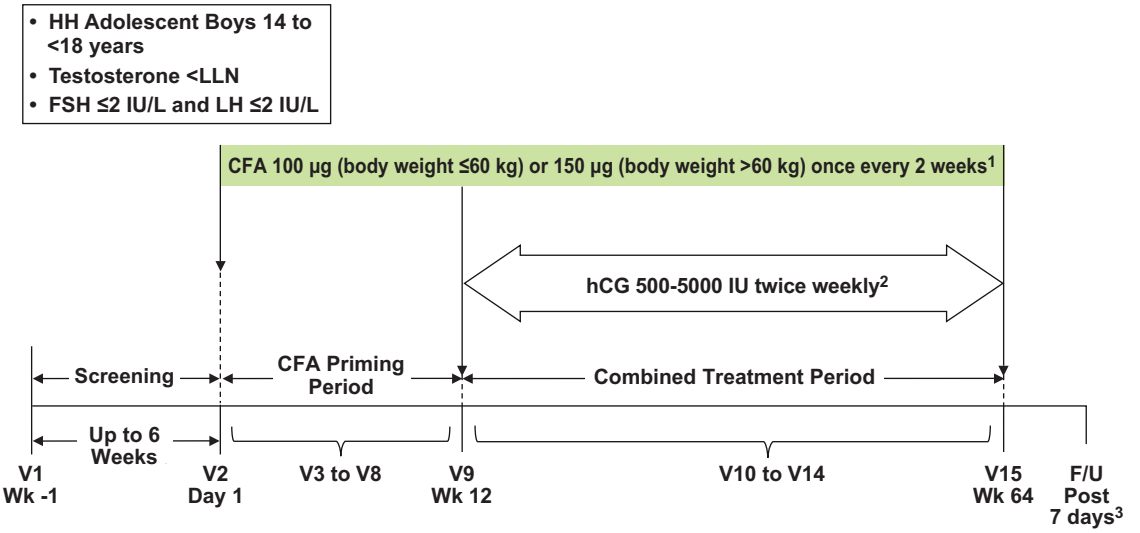
This was a 64-week, multicenter, open label, single-group study of CFA in adolescent boys aged 14 to younger than 18 years with HH (Merck protocol 043; EudraCT:

2015-001878-18). The trial design diagram is shown in Fig. 1. Individuals underwent a screening period of up to 6 weeks. Each participant and his legal guardian (or someone designated by the participant's legal guardian, such as a caregiver or parent) were given instructions on CFA and hCG home-injection before the initiation of treatment.

Eligible participants were allocated to a 12-week CFA priming period at baseline. This study used the same formulation of CFA developed and available commercially for controlled ovarian stimulation in women. Starting at baseline, a single dose of CFA (100 µg if body weight was ≤ 60 kg, or 150 µg if body weight was > 60 kg) was injected subcutaneously in the abdominal wall once every 2 weeks in the morning on the same day of the week throughout the 64-week trial. The first 3 doses of CFA were to be self-administered by the participant (or administered by an appropriately trained person designated by the participant's legal guardian) at the trial site, witnessed by qualified personnel. Participants who received CFA 100 µg at the start of the trial had their CFA dose increased to 150 µg if their body weight increased by 2 kg or more from the previous visit to a value greater than 60 kg. For participants receiving CFA 150 µg, the dose was not down-titrated for the rest of the trial, regardless of changes in body weight. A telephone contact was conducted at week 8 to monitor adverse events, concomitant medication, and study medication compliance.

At week 12, participants entered a 52-week combined treatment period during which CFA was coadministered with hCG (PREGNYL; Merck & Co Inc). Starting from the last day of week 12, hCG was injected subcutaneously twice a week in the morning, on fixed days of the week throughout the trial. The dose of hCG was adjusted between 500 IU and 5000 IU (inclusive) to keep the total T and estradiol (E2) levels within ranges specified in the protocol based on the normative data for the central laboratory (8.3-33.0 nmol/L for T; < 40% of upper limit of normal [< 147 pmol/L] for E2). The first dose of hCG at week 12 was self-administered (or administered by an appropriately trained person designated by the participant's legal guardian) at the trial site as a witnessed dose. During the combined treatment period, participants had clinic visits every 8 to 12 weeks from week 12 until treatment completion at week 64. Telephone contacts were conducted every 4 weeks between site visits to monitor adverse events, concomitant medication, and study medication compliance. The last dose of CFA was scheduled to be home-administered by the participant (or an appropriately trained person designated by the participant's legal guardian) at week 62. The participant (or the designated person) continued to home-administer the twice-weekly hCG, and the last dose was administered at week 64. A posttreatment follow-up visit was conducted at least 21 days (but ≤ 45 days) after the last dose of CFA, and at least 7 days after the last dose of hCG (even if study treatment was discontinued prematurely). The overall treatment period was defined as the period from the first dose of CFA to the completion of the posttreatment follow-up visit.

Blood samples for serum FSH, LH, hCG, inhibin-B, antimüllerian hormone (AMH), free T, and sex hormone–binding globulin (SHBG) measurements were collected at screening, baseline, weeks 12, 36, 60, and 64, and a posttreatment follow-up visit at least 21 days (but ≤ 45 days)



1. During the course of the trial, for subjects receiving CFA 100 µg, the dose of CFA was adjusted to 150 µg if the subject's body weight increased by 2 kg or more from the previous visit to a value greater than 60 kg. For subjects receiving CFA 150 µg, the dose was not down-titrated for the rest of the trial regardless of any changes in body weight.
2. During the Combined Treatment Phase, the dose of hCG was titrated between 500-5000 IU twice weekly to keep the testosterone and E2 levels within acceptable ranges.
3. Follow-up visit was conducted at least 21 days (but no more than 45 days) after the last dose of CFA and at least 7 days after the last dose of hCG.

Abbreviations: CFA, corifollitropin alfa; F/U, follow-up visit; FSH, follicle stimulating hormone; hCG, human chorionic gonadotropin; HH, hypogonadotropic hypogonadism; LH, luteinizing hormone; LLN, lower limit of normal.

Figure 1. Trial design diagram.

after the last dose of CFA and at least 7 days after the last dose of hCG. Blood samples for serum total T and E2 measurements were collected at screening, baseline, weeks 12, 16, 24, 36, 48, 56, and 64, and the posttreatment follow-up visit. Blood samples for serum CFA measurements were collected at baseline, weeks 2, 4, 12, 24, 36, 48, and 64, and the posttreatment follow-up visit. Blood samples for serum antidrug antibody (ADA) measurements were collected at baseline, weeks 4, 12, 24, 36, 48, and 60, and the posttreatment follow-up visit.

Efficacy End Points

The primary efficacy measure was TV (ie, the sum of left and right testes volumes), which was measured using ultrasound at weeks 1, 2, 12, 36, and 64. All ultrasound images were evaluated locally for pathological findings at all time points by a qualified investigator or radiologist, and centrally by a radiologist at a blinded, sponsor-validated imaging vendor. In addition, at screening, TV was calculated locally to determine eligibility for enrollment and to assess for any pathology that would have excluded the patients from participating in the study. The sponsor-validated central imaging vendor read all testicular ultrasound images after the participants had completed the study with the order of the images masked. The volume of each testis was calculated by the radiologist at the central imaging vendor using the formula for a prolate ellipsoid: $(\pi/6) \times$ (longitudinal) $\times$ (anteroposterior) $\times$ (transverse) diameters.

Secondary efficacy end points included Tanner stage of development, growth velocity, and hormonal markers for puberty (T, inhibin B, AMH, E2, LH, FSH, and SHBG). Tanner staging assessed genital development (eg, TV and penile development) and pubic hair distribution. Tanner stage of development and growth velocity are well-recognized markers of pubertal progress.

One of the primary goals in treating participants with HH is to increase T levels; both total and free T were evaluated. In males, increases in E2 signify aromatization of T, and decreases in SHBG are a response to T and changes in the T/E2 ratio in favor of T.

Direct assessment of spermatogenesis was not conducted in this study because of the age of the participants. Inhibin B was measured because it has been shown to be a useful surrogate for monitoring spermatogenic activity in boys treated with hCG when semen analysis is not feasible (6).

Serum hCG levels were measured to monitor treatment compliance.

Hormone and Sex Hormone–binding Globulin Assays

After solvent extraction, total serum T was measured using liquid chromatography–tandem mass spectrometry with a QTRAP 5500 mass spectrometer (SCIEX). The assay sensitivity was 0.07 nmol/L. The intra-assay and interassay precision measures (expressed as % coefficient of variation [% CV]) for this assay were 1.3% to 2.4% CV and 2.1% to 5.1% CV, respectively. After equilibrium dialysis and liquid extraction, free T concentration was determined using liquid chromatography with tandem mass spectrometric detection with a QTRAP 5500 mass spectrometer. The assay sensitivity was 69.3 pmol/L. The intra-assay and interassay precision measures for this assay were 4.9% to 5.8% CV and 5.3% to 8.8% CV, respectively.

Inhibin B was measured using a qualitative enzyme-linked immunosorbent assay (INHIBIN B GEN II ELISA; Beckman Coulter; RRID: AB_2827405; catalog No. A81301). The assay sensitivity was 10 ng/L. The intra-assay and interassay precision measures for this assay were 4.0% CV and 3.5% to 5.1% CV, respectively.

AMH was measured using a sandwich principle electrochemiluminescence immunoassay (Elecsys AMH assay; Roche Diagnostics; RRID: AB_2895131; catalog No. 06331076). The assay sensitivity was 0.21 pmol/L. The intra-assay and interassay precision measures for this assay were 0.5% CV and 1.5% to 1.8% CV, respectively.

E2 was measured using a competitive assay (ADVIA Centaur Enhanced Estradiol assay; Siemens; RRID: AB_2895133; catalog No. 10490889). The assay sensitivity was 69.80 pmol/L. The intra-assay and interassay precision measures for this assay were 2.2% to 3.9% CV and 2.7% to 4.8% CV, respectively.

LH was measured using a sequential, 2-step immunoenzymatic assay (Access hLH assay; Beckman-Coulter; RRID: AB_2750984; catalog No. 33510) with a DxI 800 luminometer (Beckman-Coulter). The assay sensitivity was 0.20 IU/L. The intra-assay and interassay precision measures for this assay were 3.0% to 4.3% CV and 3.9% to 6.7% CV, respectively.

FSH was measured using a sequential 2-step immunoenzymatic assay (Access hFSH assay; Beckman-Coulter; RRID: AB_2750983; catalog No. 33520) with a DxI 800 luminometer (Beckman-Coulter). The assay sensitivity was 0.60 IU/L. The intra-assay and interassay precision measures for this assay were 3.8% to 4.7% CV and 5.0% to 6.1% CV, respectively.

SHBG was measured using a chemiluminescent assay (Siemens; RRID: AB_2819251; catalog No. L2KSH-20) with an Immulite 2000/2000XPi luminometer (Siemens). The assay sensitivity was 2.0 nmol/L. The intra-assay and interassay precision measures for this assay were 4.1% to 4.5% CV and 3.1% to 6.9% CV, respectively.

hCG was measured using a sandwich principle electrochemiluminescence immunoassay (Roche Diagnostics; RRID: AB_2895132; catalog No. 03271749). The assay sensitivity was 0.6 IU/L. The intra-assay and interassay precision measures for this assay were 1.1% CV and 2.1% to 3.1% CV, respectively.

Pharmacokinetic and Immunogenicity Assessments

Serum CFA concentrations were determined using an indirect enzyme-linked immunosorbent assay (ELISA; Merck & Co Inc; RRID: AB_2895553; catalog No. SB_1,) with a SpectraMax M5 (Molecular Devices Corp). The assay sensitivity was 312.50 pg/mL and the intra-assay and interassay precision measures for this assay were 1.0% to 12.3% CV and 4.2% to 7.9% CV, respectively.

Serum samples collected for ADA assay were analyzed in a 2-step process. The initial screening assay used a radioimmunoassay (Merck & Co Inc; RRID: AB_2895554; catalog No. SB_2) in which the samples were incubated overnight with ^{125}I-CFA. Samples found to be positive for ADA in the screening assay were subjected to a confirmatory immunodepletion assay for which the samples were incubated both with ^{125}I-CFA and nonlabeled CFA. The assay sensitivity was 3.250 ng/mL and the intra-assay and interassay precision measures for this assay were 0.1% to 16.5% CV and 5.1% to 17.3% CV, respectively.

Safety Assessments

Safety and tolerability were assessed by clinical review of adverse events, presence of CFA antibodies, laboratory tests, and vital signs. The prespecified safety end point was evaluation of development of antibodies to CFA.

Statistical Methods

The primary analyses for efficacy were based on the full analysis set (FAS) population, which consisted of all participants who had a baseline and at least 1 postbaseline measurement of TV, had no LH levels greater than 3 IU/L, had received treatment for at least 12 weeks with CFA followed by 24 weeks of CFA and hCG, and had no more than 4 weeks from the last dose of CFA when the last TV measurement was made.

The primary efficacy end point was the mean change from baseline in log-transformed TV at week 64, which was analyzed using a mixed model with a fixed effect for baseline and time point and a random effect for each participant. The geometric mean increase in TV and its 95% CIs were obtained by back transformation to natural scale by exponentiation. The mean change from the first day of combined treatment (week 12) to week 64 in log-transformed TV was also analyzed using a mixed model with a fixed effect for time point. For each time point, the mean change from week 12 to that time point and the associated 95% CI were calculated. The geometric mean increase in TV and its 95% CI were obtained by exponentiation.

The analyses for all secondary efficacy end points were conducted on the FAS population. The change in serum inhibin B concentrations after weeks 12, 36, and 64 were summarized. Growth velocity over the 36- and 64-week treatment periods was extracted using the slopes estimated from an overall mixed random intercept and random slope model of height (cm) and time (years) and age as covariates. Tanner staging was recorded for both TV and pubic hair at baseline, and weeks 12, 36, and 64. Serum concentrations of hormones (total T, inhibin B, AMH, E2, LH, and FSH) and SHBG were summarized by assessment. Sonographic testicular patterns were listed and any changes over time were described.

Descriptive summary statistical analysis of CFA serum concentrations was conducted using the software Phoenix WinNonlin Professional (version 8.1.). Relative nominal pharmacokinetic sampling time was used for this analysis. Values below the serum CFA assay lower limit of quantitation were replaced with 0. Descriptive statistics for the serum concentrations by time point were calculated.

Safety analyses were performed in the all subjects as treated (ASaT) population consisting of all participants who received at least one dose of CFA.

Results

This study was conducted from February 2, 2017 through May 5, 2020. Seventeen participants were enrolled in the study. All 17 participants completed the CFA priming period and entered the combined CFA and hCG treatment period. One participant discontinued study medication during the combined treatment period because of a serious adverse event (recurrence of craniopharyngioma) but continued in the study. All 17 of the enrolled participants completed the study. Of the 17 participants, 13 were included in the FAS population for the efficacy analyses. Four participants, including 1 whose last TV measurement was made more than 4 weeks after the last CFA dose, 1 who did not have baseline TV data, and 2 who did not have LH measurements at week 64, could not be included in the FAS population.

Demographics and Baseline Characteristics

Table 1 summarizes the demographic and baseline disease characteristics of the overall study population. All participants were prepubertal adolescent boys, with a mean age of 15.5 years, a mean height of 161.4 cm, and a mean TV of 2.2 mL. Genitalia were prepubertal (with the exception of one participant with Tanner II genitalia), as were the levels of FSH, LH, and total T. Pubic hair showed more variability (Tanner stage I to III).

Corifollitropin Alfa Serum Concentration

Table 2 summarizes mean (SD) CFA serum concentration over time for the CFA priming period and the combined

Table 1. Demographics and baseline disease characteristics

Parameter	
Participants, No.	17
Male, n (%)	17 (100)
Mean (SD) age, y	15.5 (0.9)
Race, n (%)	1 (5.9)
Multiple	1 (5.9)
Black or African American, White	16 (94.1)
White	
Ethnicity	4 (23.5)
Hispanic or Latino	13 (76.5)
Not Hispanic or Latino	
Etiology of gonadotropin deficiency	1 (5.9)
CHARGE syndrome	1 (5.9)
Kallmann syndrome	14 (82.4)
Hypogonadotropic hypogonadism without olfaction deficit/olfactive bulb hypoplasia	1 (5.9)
Unknown[a]	
Tanner stage—pubic hair, n (%)	7 (41.2)
Tanner I	9 (52.9)
Tanner II	1 (5.9)
Tanner III	
Tanner stage—genitalia, n (%)	16 (94.1)
Tanner I	1 (5.9)
Tanner II	
Mean (SD) height, cm	161.4 (11.2)
Mean (SD) weight, kg	57.7 (16.6)
Mean (SD) BMI	21.9 (4.9)
Mean (SD) testicular volume, mL	2.2 (1.9)
Mean (SD) follicle-stimulating hormone, IU/L	0.5 (0.4)
Mean (SD) luteinizing hormone, IU/L	0.2 (0.2)
Mean (SD) human chorionic gonadotropin, choriogonadotropin β, IU/L	0.3 (0.0)
Mean (SD) total testosterone, nmol/L	0.3 (0.4)
Mean (SD) estradiol, pmol/L	37.1 (8.9)
Mean (SD) sex hormone–binding globulin, nmol/L	55.3(31.3)
Mean (SD) inhibin B, ng/L	44.9 (46.5)
Mean (SD) antimüllerian hormone, pmol/L	183.8 (90.0)

Abbreviation: BMI, body mass index; CHARGE, coloboma of the eye, heart anomaly, choanal atresia, retardation, and genital and ear anomalies.
[a]Due to missing data.

Table 2. Mean (SD) corifollitropin alfa serum concentration over time

Study period	Treatment	Sampling time	No.	Mean (SD) (ng/L)
Priming period	CFA	Pre first dose	16[a]	0 (0)
		6-24 h post first dose	16[a]	4100 (2630)
		32-52 h post first dose	17	5880 (1640)
		72-120 h post first dose	17	4240 (1180)
		144-192 h post first dose	16[b]	2110 (864)
		216-264 h post first dose	17	1150 (655)
		Predose to 3rd CFA injection	16[c]	480 (348)
		Predose to 7th CFA injection	17	695 (1310)
Combined treatment period	CFA with hCG	Predose to 9th CFA injection	17	691 (1060)
		Predose to 13th CFA injection	17	301 (297)
		Predose to 25th (last) CFA injection	16[d]	556 (1260)
		Post last CFA injection	16[d]	864 (1290)
		Follow-up	16[d]	0 (0)

Abbreviations: CFA, corifollitropin alfa; hCG, human chorionic gonadotropin.
[a]A predose and 6- to 24-h postdose serum sample from one participant were excluded from the descriptive summary statistical analysis because of a biological implausibility.
[b]A serum sample from one participant was not collected at visit 5.
[c]A serum sample from one participant collected at visit 8 was not considered evaluable and was excluded from the descriptive summary statistical analysis.
[d]One participant discontinued study medication before last CFA injection.

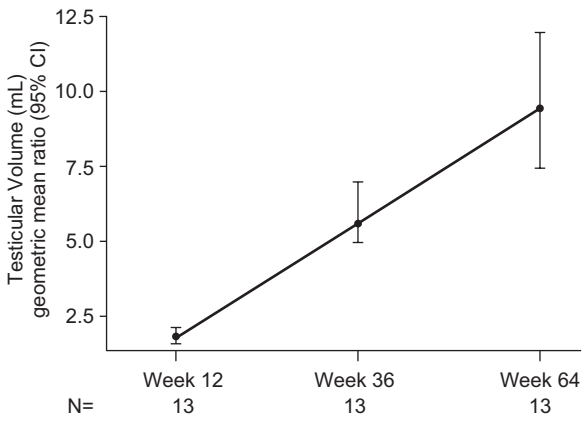


Figure 2. Model-based geometric mean (95% CI) testicular volume change over time (full analysis set population). Testicular volume was determined as the sum of left and right testes volumes, evaluated using ultrasound.

treatment period. After the first CFA dose, the highest mean (SD) CFA serum concentrations were observed in the first 2 days postdose and declined slowly over the dosing interval. After repeated CFA dosing, mean predose (trough) CFA serum concentrations were generally similar for CFA alone (priming period) and CFA coadministered with hCG (combined treatment period), ranging from 301 ng/L to 864 ng/L. CFA serum concentrations were comparable in participants receiving 100 µg and 150 µg doses of CFA (data not shown).

Testicular Development

In the CFA priming period, for the FAS population, the mean TV increased from a geometric mean of 1.4 mL (arithmetic mean, 1.5 mL) at baseline to 2.5 mL (arithmetic mean, 2.8 mL) at week 12, with a geometric mean fold increase of 1.83 (95% CI, 1.58-2.13) (arithmetic mean of change from baseline at week 12, 1.3 mL). As shown in Fig. 2, during the overall treatment period, there was a continuous increase in mean TV. At baseline, the geometric mean TV was 1.4 mL (arithmetic mean, 1.5 mL; left testis, geometric mean, 0.7 mL [arithmetic mean, 0.7 mL]; right testis, geometric mean, 0.7 mL [arithmetic mean, 0.8 mL]). At week 64, the geometric mean TV was 12.9 mL (arithmetic mean, 14.5 mL) and the estimated geometric mean fold increase from baseline was 9.43 (95% CI, 7.44-11.97) (arithmetic mean of change from baseline at week 64, 13.0 mL). The increase in mean TV during the overall treatment period was symmetric in both testes (geometric mean TV at week 64 was 6.5 mL [arithmetic mean, 7.2 mL] for the left testis and 6.4 mL [arithmetic mean, 7.3 mL] for the right testis; estimated geometric mean fold increase from baseline at week 64 was 9.51 [95% CI, 7.38-12.27] [arithmetic mean of change from baseline at week 64, 6.5 mL] for the left testis and 9.43 [95% CI, 7.54-11.81] [arithmetic mean of change from baseline at week 64, 6.5 mL] for the right testis). Participants who began the study weighing 60 kg or less on CFA 100 µg and those who began the study weighing more than 60 kg on CFA 150 µg had similar increases in mean TV (arithmetic mean TV at week 64 was 13.7 mL for participants who began the study weighing ≤ 60 kg and 15.6 mL for those who began the study weighing > 60 kg).

Anthropometric Measures and Growth Velocity

Participants in the FAS population had a mean (SD) height at baseline of 160.6 (10.5) cm. At the end of the CFA priming period, mean (SD) height in the FAS population was 162.1 (10.3) cm. At the end of the study, mean (SD) height in the FAS population was 169.3 (8.4) cm.

Participants demonstrated increased growth velocity in response to hCG, indicative of pubertal changes. The model-based linear growth velocity (SD) in the FAS population at week 36 and week 64 was 8.3 (3.7) cm/year and 7.6 (3.5) cm/year, respectively.

Tanner Staging

Fig. 3 shows the proportion of participants in the FAS population in each Tanner stage over time for genital growth. At

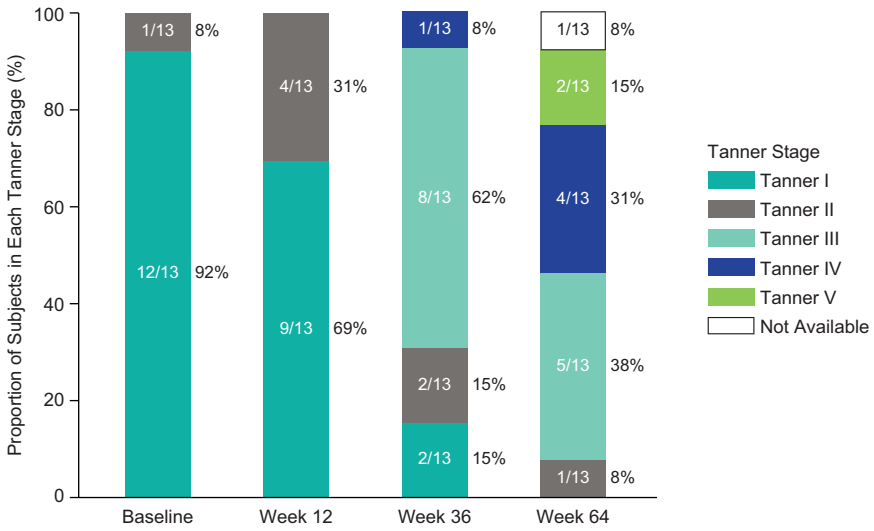


Figure 3. Proportion of participants in each Tanner stage over time for genital growth (full analysis set population). At Week 64, one participant had a genital assessment that was performed outside the analysis window and was therefore excluded from the full analysis set for that time point.

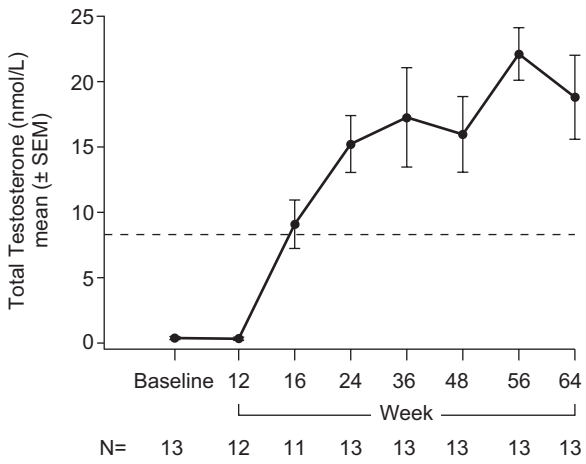


Figure 4. Mean (±SEM) total testosterone (µg/L) over time (full analysis set population). Dashed line indicates lower limit of normal for total testosterone in adolescent boys.

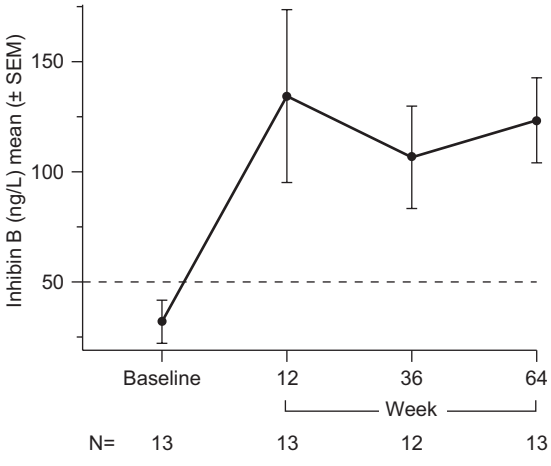


Figure 5. Mean (±SEM) inhibin B (ng/L) over time (full analysis set population). Dashed line indicates lower limit of normal for inhibin B in adolescent boys.

the start of the study, 12 (92.3%) of the 13 participants in the FAS population were prepubertal (Tanner I) and 1 participant was in early puberty (Tanner II) for genital growth. At week 36, most of the participants had progressed and were in mid puberty (Tanner III). At week 64, 1 participant was in early puberty (Tanner II), 5 participants were in mid puberty (Tanner III), and 6 participants were in late puberty (Tanner IV/V).

Pubic hair staging was more variable than genital growth at baseline. At week 64, all 13 participants in the FAS population had pubic hair development ranging from Tanner III to V (data not shown).

Hormonal and Sex Hormone–binding Globulin Responses

Fig. 4 summarizes mean (SEM) total T concentration over time for the FAS population. Mean total T concentration increased in response to hCG treatment, starting from week 12. The mean (SD) total T concentration at week 64 was 18.82 (11.60) nmol/L. Free T concentration also increased in response to hCG treatment, starting from week 12 (data not shown); the mean (SD) free T concentration for the FAS analysis population at week 64 was 388.44 (240.84) pmol/L.

Fig. 5 summarizes mean (SEM) inhibin B concentration over time for the FAS population. There was a mean increase in inhibin B concentration at week 12 in response to CFA that was maintained through week 64.

Fig. 6 summarizes mean (SEM) AMH concentration over time for the FAS population. There was an initial increase in mean AMH concentration during the priming period, consistent with Sertoli cell stimulation by CFA alone. After week 12, when CFA was combined with hCG, the mean AMH concentration decreased over time, with the lowest mean AMH concentration being observed at week 64.

Mean E2 concentration increased in response to hCG treatment, starting from week 12 (data not shown). The mean (SD) E2 concentration in the FAS population at week 64 was 202.17 (150.79) pmol/L.

There was no evidence of development of spontaneous puberty (ie, LH > 3 IU/L) in any participant in the FAS population during the study. Mean (SD) LH levels in the FAS population were 0.25 (0.24), 0.17 (0.13), and 0.12 (0.06)

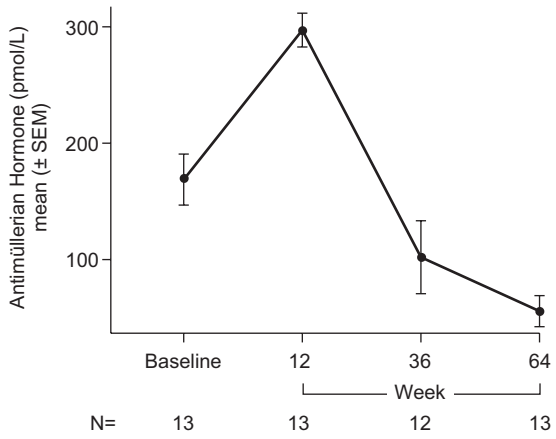


Figure 6. Mean (±SEM) antimüllerian hormone (µg/L) over time (full analysis set population).

IU/L at baseline, week 12, and week 64, respectively. Mean (SD) FSH levels in the FAS population were 0.56 (0.45), 1.31 (2.03), and 1.53 (1.86) IU/L at baseline, week 12, and week 64, respectively.

At baseline, the mean (SD) SHBG concentration in the FAS population was 53.19 (31.68) nmol/L. At week 12, the mean (SD) SHBG concentration in the FAS population was 54.56 (31.90) nmol/L. At week 64, the mean (SD) SHBG concentration in the FAS population had decreased to 29.47 (15.11) nmol/L.

Safety

A total of 16 (94.1%) of the 17 study participants were reported to have had 1 or more adverse events. In the CFA priming period, the most frequently reported adverse events were headache (3/17 [17.6%]) and rhinorrhea (2/17 [11.8%]). In the combined treatment period, the most frequently reported adverse events were spermatocele (5/17 [29.4%]), increased E2 (5/17 [29.4%]), and increased blood T (4/17 [23.5%]). Drug-related adverse events (as assessed by the investigator) were reported for 9 (52.9%) of the 17 study participants: 7 (41.2%) participants had drug-related adverse events related to hCG that included increased blood T and increased E2 (1 report of increased E2 was related to an accidental hCG overdose); 1 participant had a drug-related adverse event related to CFA (hot flush); and 1 participant had drug-related adverse events related to CFA (vomiting and injection site pain) as well as a drug-related adverse event related to both CFA and hCG (acne). No clinically relevant changes in laboratory values or vital signs were observed. One participant with a previous medical history of craniopharyngioma had a serious adverse event of recurrent craniopharyngioma. This serious adverse event led to discontinuation of study medication and was assessed by the investigator not to be related to the study medication. The event was treated, and the participant completed the study off the study medication. No participant developed confirmed anti-CFA antibodies in this study. No adverse event of gynecomastia was reported for any of the participants.

Discussion

The present study in prepubertal adolescent boys aged 14 to younger than 18 years with HH demonstrated that treatment with CFA (alone for 12 weeks and then combined with hCG for weeks 12-64) was well tolerated and effective for the induction of pubertal development. Improvements in TV, Tanner stages (genitalia and pubic hair), height, and hormonal parameters were consistent with changes typical of normally timed puberty in boys without HH. These results are consistent with the therapeutic benefits reported in previous studies with recFSH and hCG in adolescent boys with HH (2, 11-16).

Testes grow throughout childhood, and mean volume (assessed by a Prader orchidometer) is typically greater than 3 mL at the onset of normally timed puberty (21-26). More recently, using ultrasound, the mean TV before the onset of puberty in boys with normally timed puberty was determined to be 0.6 mL (ie, volume of ~1.2 mL for TV measured as the sum of volumes of the right and left testes) (25). Treatment in adolescent boys with HH is typically delayed beyond the onset of normally timed puberty. Thus, baseline TV in these patients is likely to be greater than 1.2 mL, reflecting continued prepubertal growth before the start of treatment (14). Indeed, in the present study, in which the mean age at baseline was 15.5 years, mean TV at baseline for the 17 study participants was 2.2 mL. During the treatment period, there was a continuous increase in the primary end point measure of mean TV (measured by ultrasound), with the largest increase observed at week 64. For the FAS population, the geometric mean TV achieved at the end of week 64 was 12.9 mL (arithmetic mean, 14.5 mL), which was less than the mean TV observed at completion of normally timed puberty in adolescent boys (14). This difference probably reflects that the participants in the present study were exposed to CFA/CFA + hCG therapy for only 64 weeks, while it generally takes up to 2 to 3 years for completion of puberty in adolescent boys (14).

Participants demonstrated increased linear growth in response to hCG, indicative of pubertal development. Growth velocity at week 36 (8.3 cm/year) and week 64 (7.6 cm/year) was as expected in adolescent boys with HH responding to hCG therapy. Mean (SD) height at baseline in the FAS population was 160.1 (10.5) cm. Adolescent boys undergoing normally timed puberty have been reported to have a mean prepubertal height of 156 cm at age 13 (27). The greater mean baseline height observed in the present study reflected the fact that the participants were older than 13 years at baseline and therefore had undergone further prepubertal growth. At the end of the study, mean (SD) height was 169.3 (8.4) cm. The changes in growth velocity and height observed in the present study were generally consistent with those reported in boys during the first year of normally timed puberty (27).

Mean total T and free T concentrations increased in response to hCG treatment, starting from week 12. The mean (SD) total T concentration at week 64 (18.82 [11.60] nmol/L) was within the normal range (3.5-41.6 nmol/L) observed in adolescent boys undergoing normally timed puberty. While normalizing T levels with T supplementation alone may not improve indices such as health-related QoL even after 2 years of therapy (28), adolescent boys treated with gonadotropins for HH show significant improvements in QoL and self-reported satisfaction scores (15).

The increase in E2 serum concentration observed in response to hCG treatment was consistent with the changes in total T levels and reflect peripheral aromatization of the

hCG-induced increased T. No participant reported an adverse event of gynecomastia.

There was a mean increase in inhibin B at week 12, which was maintained through week 64. The observed increase in inhibin B was consistent with proliferation of Sertoli cells in response to CFA. The number of Sertoli cells is associated with sperm-producing capacity (2). There was an initial increase in AMH during the priming period, which is consistent with stimulation by CFA alone (29). After week 12, when CFA was combined with hCG, mean AMH levels decreased, with the lowest AMH value occurring at week 64. Taken together, the inhibin-B and AMH levels suggest that the testes of these participants had matured—the production of T in response to hCG led to a transition from AMH to inhibin B production in Sertoli cells (marking the transition from proliferation to maturation of Sertoli cells), a finding associated with puberty.

The production of SHBG in the liver is regulated by the ratio of T and E2 levels, in addition to other factors, including thyroid hormone status, dietary factors, certain diseases, and medications (30). In boys undergoing puberty, a change in the T/E2 ratio in favor of T leads to a decrease in SHBG levels, as was observed in the present study in response to treatment with hCG.

During the course of the study, endogenous LH was expected to remain low. However, if the diagnosis of HH was incorrect, and the participant had constitutional delay of growth and puberty instead, such a participant could undergo spontaneous puberty, as indicated by increases in LH levels to greater than 3 IU/L. Mean LH and FSH levels remained low throughout the study. No participant included in the FAS population had evidence of spontaneous puberty. Two participants had missing LH measurements at week 64. The possibility that these 2 participants may have undergone spontaneous puberty during the study could not be precluded; therefore, they were excluded from the FAS population. These participants did not appear to be different from the 13 participants included in the FAS population in terms of their baseline TV or the response to treatment at week 64 (data not shown).

This study was not designed to test for spontaneous reversal of HH; as described previously (31, 32), approximately 10% to 15% of patients with HH of varied etiologies may have spontaneous reversal of HH lasting for varying periods of time. After differing durations of treatment for virilization or fertility induction, these patients maintain TV, T, and inhibin B levels even after interruption of therapy; reversible HH is characterized by activation of the hypothalamic-pituitary-gonadal axis with normalization of downstream endocrine actions. In the one participant in this study who discontinued CFA and hCG following the diagnosis of recurrence of craniopharyngioma, changes in TV, T levels, and inhibin B levels observed while he was on treatment were not sustained after discontinuation.

Treatment with CFA (alone for 12 weeks and then combined with hCG for weeks 12-64) was generally well tolerated. No participant developed confirmed anti-CFA antibodies. Adverse events reported in these participants did not alter the known safety profile of CFA in women (19) and men (20). While FSH receptors have been reported to be expressed on the surface of blood vessels in tumors (33), currently, there is no definitive evidence linking the use of gonadotropins for replacement therapy with de novo tumor development (19, 34).

Limitations of this study include the small number of participants; however, since HH is a rare condition, enrollment in larger studies would be more difficult. The study was also not placebo controlled; however, inclusion of a placebo group would have increased sample size, making recruitment more difficult. Further, a placebo group would mean that those assigned to placebo would not have received treatment during a potentially critical window of time, and while inclusion of a placebo group would be more important if the likelihood of enrolling adolescent boys with constitutional delay was high, the inclusion criteria for this study (eg, the requirement that patients be aged ≥ 14 years) made this possibility very unlikely, and LH was assessed throughout the study and participants in the FAS population showed no evidence of spontaneous puberty. Therefore, the addition of a placebo arm would have been unlikely to alter the assessment of safety and efficacy of CFA in the studied population. Similarly, the study did not include an active control group. The recruitment of adolescent boys with HH for this study was difficult; it took approximately 2 years to recruit the 17 patients who participated in this study. The inclusion of a small control group would have increased the sample size, which would have made recruitment even more challenging. Moreover, given the small population available to enter the trial, the robustness of any comparison to an active control such as T or recFSH would have been very limited in the absence of a study designed to assess noninferiority. Given these challenges to recruitment, a single-arm study was considered appropriate, though not ideal, particularly in view of the evidence for the CFA activity at the FSH receptor and the prior reports of response to FSH in this population. Gonadotropin therapy (CFA alone and then combined with hCG) was examined for 64 weeks in this study, which was not long enough to allow for completion of puberty; generally, gonadotropin treatment needs to be administered for at least 2 to 3 years to mimic the events in boys without HH to achieve completion of puberty. However, there is no reason to expect that the response in TV and other markers of puberty observed in these participants over 64 weeks will not be continued if the duration of therapy were extended for 2 to 3 years, allowing for pubertal completion. Last, the exclusion of adolescents with HH and cryptorchidism may have introduced a potential positive bias by excluding those patients with a primary testicular defect, leading to poorer responses to gonadotropin therapy (35).

In conclusion, treatment with CFA (alone for 12 weeks and then combined with hCG for weeks 12-64) was generally well tolerated and demonstrated benefit for the induction of testicular growth and normal pubertal development in adolescent boys with HH. These results suggest that CFA can replace recFSH as an option involving fewer injections in the treatment of adolescent boys with HH in whom support of spermatogenesis is an important component of the desired outcome. Furthermore, fewer injections with CFA are needed compared with a treatment regimen that uses recFSH, and this may decrease treatment burden and improve adherence, a benefit considered particularly attractive for an adolescent male population.

Acknowledgments

We would like to thank Alan Meehan for editorial assistance and Jennifer Rotonda and Michele McColgan for assistance

in preparing the manuscript for submission. We also would like to thank Rebecca Wrishko and Sucharita Bhattacharyya for coordinating and securing the serum CFA and ADA bioanalysis data from the vendor (Syneos) and evaluating the serum CFA and ADA bioanalysis data. Alan Meehan, Jennifer Rotonda, Michele McColgan, Rebecca Wrishko, and Sucharita Bhattacharyya are employees of Merck Sharp & Dohme Corp, a subsidiary of Merck & Co, Kenilworth, New Jersey, USA.

Financial Support

This work was supported by Merck Sharp & Dohme Corp, a subsidiary of Merck & Co, Inc, Kenilworth, New Jersey, USA.

Author Contributions

All authors provided final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. As per ICMJE rules, all authors contributed to the conception, design or planning; acquisition/analysis of the data; and/or interpretation of the results. They also drafted the manuscript and/or critically reviewed the manuscript for important intellectual content.

Disclosures

R.R.S., S.S., H.-K.J., G.M., J.R.D., Y.G., B.J.S., M.C.F., and K.D.K. are current or former employees of Merck Sharp & Dohme Corp, a subsidiary of Merck & Co, Inc, Kenilworth, New Jersey, USA, or of Organon & Co, Inc, Jersey City, New Jersey, USA, and may own stock/stock options in Merck & Co, Inc, Kenilworth, New Jersey, USA, or in Organon & Co, Inc, Jersey City, New Jersey, USA. E.N., H.M.B., and R.S.S. have nothing to disclose.

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided. The data-sharing policy, including restrictions, of Merck Sharp & Dohme Corp, a subsidiary of Merck & Co, Inc, Kenilworth, New Jersey, USA, is available at http://engagezone.msd.com/ds_documentation.php. Requests for access to the clinical study data can be submitted through the EngageZone site or via email to dataaccess@merck.com.

Clinical Trial Information

EudraCT registration number 2015-001878-18 (registered 2018-01-11).

References

1. Fraietta R, Zylberstejn DS, Esteves SC. Hypogonadotropic hypogonadism revisited. *Clinics (Sao Paulo)* 2013;68(Suppl 1):81-88.
2. Raivio T, Wikström AM, Dunkel L. Treatment of gonadotropin-deficient boys with recombinant human FSH: long-term observation and outcome. *Eur J Endocrinol.* 2007;156(1):105-111.
3. Dwyer AA, Sykiotis GP, Hayes FJ, *et al*. Trial of recombinant follicle-stimulating hormone pretreatment for GnRH-induced fertility in patients with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab.* 2013;98(11):E1790-E1795.
4. Aydogan U, Aydogdu A, Akbulut H, *et al*. Increased frequency of anxiety, depression, quality of life and sexual life in young hypogonadotropic hypogonadal males and impacts of testosterone replacement therapy on these conditions. *Endocr J.* 2012;59(12):1099-1105.
5. Petak SM, Nankin HR, Spark RF, Swerdloff RS, Rodriguez-Rigau LJ; American Association of Clinical Endocrinologists. American Association of Clinical Endocrinologists Medical Guidelines for clinical practice for the evaluation and treatment of hypogonadism in adult male patients—2002 update. *Endocr Pract.* 2002;8(6):440-456.
6. Han TS, Bouloux PM. What is the optimal therapy for young males with hypogonadotropic hypogonadism? *Clin Endocrinol (Oxf).* 2010;72(6):731-737.
7. Boehm U, Bouloux P-M, Dattani MT, *et al*. European Consensus Statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol.* 2015;11(9):547-564.
8. Vicari E, Mongioì A, Calogero AE, *et al*. Therapy with human chorionic gonadotrophin alone induces spermatogenesis in men with isolated hypogonadotrophic hypogonadism—long-term follow-up. *Int J Androl.* 1992;15(4):320-329.
9. Liu PY, Turner L, Rushford D, *et al*. Efficacy and safety of recombinant human follicle stimulating hormone (Gonal-F) with urinary human chorionic gonadotrophin for induction of spermatogenesis and fertility in gonadotrophin-deficient men. *Hum Reprod.* 1999;14(6):1540-1545.
10. Liu PY, Baker HWG, Jayadev V, Zacharin M, Conway AJ, Handelsman DJ. Induction of spermatogenesis and fertility during gonadotropin treatment of gonadotropin-deficient infertile men: predictors of fertility outcome. *J Clin Endocrinol Metab.* 2009;94(3):801-808.
11. Zacharin M, Sabin MA, Nair VV, Dabadghao P. Addition of recombinant follicle-stimulating hormone to human chorionic gonadotropin treatment in adolescents and young adults with hypogonadotropic hypogonadism promotes normal testicular growth and may promote early spermatogenesis. *Fertil Steril.* 2012;98(4):836-842.
12. Dwyer AA, Raivio T, Pitteloud N. Gonadotrophin replacement for induction of fertility in hypogonadal men. *Best Pract Res Clin Endocrinol Metab.* 2015;29(1):91-103.
13. Rastrelli G, Corona G, Mannucci E, Maggi M. Factors affecting spermatogenesis upon gonadotropin-replacement therapy: a meta-analytic study. *Andrology.* 2014;2(6):794-808.
14. Koskenniemi JJ, Virtanen HE, Toppari J. Testicular growth and development in puberty. *Curr Opin Endocrinol Diabetes Obes.* 2017;24(3):215-224.
15. Rohayem J, Hauffa BP, Zacharin M, Kliesch S, Zitzmann M; German Adolescent Hypogonadotropic Hypogonadism Study Group. Testicular growth and spermatogenesis: new goals for pubertal hormone replacement in boys with hypogonadotropic hypogonadism? —a multicentre prospective study of hCG/rFSH treatment outcomes during adolescence. *Clin Endocrinol (Oxf).* 2017;86(1):75-87.
16. Barrio R, de Luis D, Alonso M, Lamas A, Moreno JC. Induction of puberty with human chorionic gonadotropin and follicle-stimulating hormone in adolescent males with hypogonadotropic hypogonadism. *Fertil Steril.* 1999;71(2):244-248.
17. Raivio T, Toppari J, Perheentupa A, McNeilly AS, Dunkel L. Treatment of prepubertal gonadotrophin-deficient boys with

recombinant human follicle-stimulating hormone. *Lancet*. 1997;350(9073):263-264.
18. Andersson AM, Juul A, Petersen JH, Müller J, Groome NP, Skakkebaek NE. Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. *J Clin Endocrinol Metab*. 1997;82(12):3976-3981.
19. ELONVA Summary of product characteristics. Accessed August 18, 2021. https://www.ema.europa.eu/en/documents/product-information/elonva-epar-product-information_en.pdf
20. Nieschlag E, Bouloux PG, Stegmann BJ, *et al*. An open-label clinical trial to investigate the efficacy and safety of corifollitropin alfa combined with hCG in adult men with hypogonadotropic hypogonadism. *Reprod Biol Endocrinol*. 2017;15(1):17.
21. Prader A. Testicular size: assessment and clinical importance. *Triangle*. 1966;7(6):240-243.
22. Biro FM, Lucky AW, Huster GA, Morrison JA. Pubertal staging in boys. *J Pediatr*. 1995;127(1):100-102.
23. Zachmann M, Prader A, Kind HP, Häfliger H, Budliger H. Testicular volume during adolescence. Cross-sectional and longitudinal studies. *Helv Paediatr Acta*. 1974;29(1):61-72.
24. Largo RH, Prader A. Pubertal development in Swiss boys. *Helv Paediatr Acta*. 1983;38(3):211-228.
25. Joustra SD, van der Plas EM, Goede J, *et al*. New reference charts for testicular volume in Dutch children and adolescents allow the calculation of standard deviation scores. *Acta Paediatr*. 2015;104(6):e271-e278.
26. Arendt LH, Ernst A, Lauridsen LLB, Brix N, Olsen J, Ramlau-Hansen CH. Timing of pubertal development in boys born with cryptorchidism and hypospadias: a nationwide cohort study. *Asian J Androl*. 2019;21(6):551-556.
27. Abbassi V. Growth and normal puberty. *Pediatrics*. 1998;102(Suppl 3):507-511.
28. Lašaitė L, Čeponis J, Preikša RT, Žilaitienė B. Effects of two-year testosterone replacement therapy on cognition, emotions and quality of life in young and middle-aged hypogonadal men. *Andrologia*. 2017;49(3). doi:10.1111/and.12633
29. Young J, Chanson P, Salenave S, *et al*. Testicular anti-mullerian hormone secretion is stimulated by recombinant human FSH in patients with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2005;90(2):724-728.
30. Hammond GL. Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action. *J Endocrinol*. 2016;230(1):R13-R25.
31. Raivio T, Falardeau J, Dwyer A, *et al*. Reversal of idiopathic hypogonadotropic hypogonadism. *N Engl J Med*. 2007;357(9):863-873.
32. Dwyer AA, Raivio T, Pitteloud N. Management of endocrine disease: reversible hypogonadotropic hypogonadism. *Eur J Endocrinol*. 2016;174(6):R267-R274.
33. Radu A, Pichon C, Camparo P, *et al*. Expression of follicle-stimulating hormone receptor in tumor blood vessels. *N Engl J Med*. 2010;363(17):1621-1630.
34. McCafferty R, Fawzy R. Miscellaneous hormones. In: Ray S, ed. *Side Effects of Drugs Annual*. 1st ed. Elsevier; 2017:447-455.
35. Sadov S, Koskenniemi JJ, Virtanen HE, *et al*. Testicular growth during puberty in boys with and without a history of congenital cryptorchidism. *J Clin Endocrinol Metab*. 2016;101(6):2570-2577.