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Clinical Research Article

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Tildacerfont in Adults With Classic Congenital Adrenal Hyperplasia: Results from Two Phase 2 Studies

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Abstract

Context: Congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD) is typically treated with lifelong supraphysiologic doses of glucocorticoids (GCs). Tildacerfont, a corticotropin-releasing factor type-1 receptor antagonist, may reduce excess androgen production, allowing for GC dose reduction.
Objective: Assess tildacerfont safety and efficacy.
Design and Setting: Two Phase 2 open-label studies.
Patients: Adults with 21OHD.
Intervention: Oral tildacerfont 200 to 1000 mg once daily (QD) (n = 10) or 100 to 200 mg twice daily (n = 9 and 7) for 2 weeks (Study 1), and 400 mg QD (n = 11) for 12 weeks (Study 2).

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Main Outcome Measure: Efficacy was evaluated by changes from baseline at 8 AM in adrenocorticotropic hormone (ACTH), 17-hydroxyprogesterone (17-OHP), and androstenedione (A4) according to baseline A4 ≤ 2× upper limit of normal (ULN) or A4 > 2× ULN. Safety was evaluated using adverse events (AEs) and laboratory assessments.

Results: In Study 1, evaluable participants with baseline A4 > 2× ULN (n = 11; 19-67 years, 55% female) had reductions from baseline in ACTH (–59.4% to –28.4%), 17-OHP (–38.3% to 0.3%), and A4 (–24.2% to –18.1%), with no clear dose response. In Study 2, participants with baseline A4 > 2× ULN (n = 5; 26-63 years, 40% female) had ~80% maximum mean reductions in biomarker levels. ACTH and A4 were normalized for 60% and 40%, respectively. In both studies, participants with baseline A4 ≤ 2× ULN maintained biomarker levels. AEs (in 53.6% of patients overall) included headache (7.1%) and upper respiratory tract infection (7.1%).

Conclusions: For patients with 21OHD, up to 12 weeks of oral tildacerfont reduced or maintained key hormone biomarkers toward normal.

Key Words: tildacerfont, CRF-receptor antagonist, congenital adrenal hyperplasia, androstenedione, adrenocorticotropic hormone, 17-hydroxyprogesterone

Classic congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) is an autosomal recessive disorder with an estimated incidence of 1:7000 to 1:18 000 live births worldwide (1-3), in which decreased adrenal biosynthesis of cortisol leads to accumulation of cortisol precursors, hyperplasia of the adrenal glands, and excess adrenal androgen synthesis (4,5). Pathogenic variants of the *CYP21A2* gene lead to dysfunction of the 21OH enzyme necessary for the biosynthesis of both corticosteroids and mineralocorticoids and to dysregulation across the hypothalamic-pituitary-adrenal axis (2,6). A decrease in adrenal cortisol production impairs negative feedback on the hypothalamic-pituitary-adrenal axis, which increases production of corticotropin-releasing factor (CRF) and, consequently, adrenocorticotropic hormone (ACTH), the primary stimulus for cortisol and 19-carbon steroid production in the adrenal cortex (7). Elevated CRF and ACTH results in excess accumulation of 17-hydroxyprogesterone (17-OHP) and other adrenal steroid precursors (1,6), which are then shunted to pathways not requiring the 21OH enzyme, leading to increased synthesis of androstenedione (A4) and other androgens (6).

Patients with CAH suffer from not only adrenal insufficiency but also the consequences of chronic adrenal-derived androgen excess including virilization, premature puberty, impaired fertility, acne, development of testicular adrenal rest tumors (TARTs) in males, and an impaired quality of life (5,8-10). Females may also experience hyperandrogenism, hirsutism, virilized genitalia, menstrual irregularities, and ovarian adrenal rests (11).

Currently, the standard therapeutic approach to downregulate the production of excess androgens in patients with CAH is to administer supraphysiologic levels of glucocorticoids (GC) (2), which provide negative feedback and variably reduce excess production of ACTH. However, supraphysiologic GC dosing may lead to significant side effects such as stunted growth, obesity, increased risk of developing type 2 diabetes, cardiovascular disease, osteoporosis, skin toxicities, gastrointestinal disorders, and reduced lifespan. GCs involve a narrow therapeutic window, and their chronic use can cause significant adverse effects while only partially addressing the hormonal imbalances associated with CAH (2,4,5,12).

Tildacerfont (SPR001; LY2371712) is a potent, selective, nonsteroidal, oral, second-generation CRF type 1 (CRF1) receptor antagonist that binds to CRF1 receptors in the pituitary gland with high affinity and reduces ACTH secretion. When administered to patients with CAH, the reduction in ACTH secretion is expected to reduce overproduction of adrenal cortisol precursors and androgens. By controlling excess adrenal androgens through an independent mechanism, tildacerfont may decrease the clinical symptoms associated with high androgen exposure and allow GC reduction to a dosing regimen nearing physiological levels, thereby reducing the adverse effects of supraphysiologic GCs.

First-generation CRF1 antagonists are highly lipophilic and have the potential for tissue accumulation resulting in a prolonged terminal half-life with potential toxicities (13,14). The high degree of lipophilicity results in unfavorable bioavailability and pharmacokinetic profiles. Tildacerfont, a second-generation CRF1 antagonist, was developed with structurally unique features that lower the lipophilicity and volume of distribution of the compound and improve solubility resulting in more predictable pharmacokinetic properties.

Two open-label Phase 2 clinical trials were conducted to assess the safety and efficacy of tildacerfont in adult patients with 21OHD (15). Study 201 was a proof-of-concept study with multiple ascending doses of tildacerfont and a 2-week treatment duration at each dose level. Study 202 evaluated tildacerfont in patients treated for up to 12 weeks at a single dose. The safety results from these studies and insights on patient populations and treatment efficacy gained through post hoc analyses are reported herein.

Materials and Methods

Ethics

Both studies were conducted in accordance with International Council for Harmonisation Good Clinical Practice guidelines and the Declaration of Helsinki principals and applicable local and federal regulations. Institutional review boards at each study site approved the protocols and informed consent forms, and all participants provided written consent before enrollment.

Study Objectives and Outcomes

The objectives of Study 201 (NCT03257462) and Study 202 (NCT03687242) were to evaluate the safety and efficacy of tildacerfont in adult participants with 21OHD. Study 201 also evaluated the pharmacokinetic parameters of tildacerfont. Efficacy was evaluated according to changes from baseline in 3 key biomarkers: ACTH, 17-OHP, and A4. Testicular ultrasounds were performed for males at baseline and end of treatment to assess possible treatment effects on existing TARTs in both studies. Safety was evaluated on an ongoing basis by monitoring for adverse events (AEs), physical examination findings, and changes in vital signs, electrocardiogram measurements, clinical laboratory values, and inventory scales for depression, anxiety, and suicidality.

Participant Eligibility Criteria

Study 201

Males and females aged ≥18 years were eligible to enroll in Study 201 if they had a documented diagnosis of 21OHD, were on a stable regimen of GC replacement therapy for ≥30 days before study entry, and had elevated 17-OHP ≥ 800 ng/dL at screening prior to their morning GC dose. A documented diagnosis of 21OHD was defined as a documented genetic mutation in the CYP21A2 enzyme consistent with a diagnosis of classic CAH or historical documentation of elevated 17-OHP prior to screening. Individuals were ineligible to participate if they had evidence of a clinically significant unstable medical condition or chronic disease within 30 days of screening, a clinically significant psychiatric disorder within 6 months before screening, or received prohibited concomitant medications (eg, rosiglitazone, strong CYP3A4 inhibitors/inducers, with the exception of GCs and birth control, and testosterone therapy) within 30 days or 5 half-lives of the first dose of the study drug or had a history of bilateral adrenalectomy or hypopituitarism. Pregnant or nursing females were excluded. Treatment with 1 or more of the following GC medications was permitted during the trial: hydrocortisone, prednisolone, methylprednisolone or prednisone, dexamethasone, or a combination of these medications. Mineralocorticoid replacement with fludrocortisone was also permitted but not required.

Study 202

Participants who completed Study 201 were eligible for Study 202 if they successfully completed Study 201 and were on a stable regimen of GC replacement therapy for ≥12 weeks before the Study 202 baseline. Tildacerfont-naïve participants were also allowed to enroll in Study 202, provided they met the same eligibility criteria as for Study 201.

Study Design and Assessments

Study 201

Study 201 was a Phase 2a, multicenter, open-label, multiple-dose, dose-escalation study. Participants were receiving a stable GC regimen and could not change their regimen during the study but were allowed to use stress doses for intercurrent illnesses as clinically indicated (ie, up to 100 mg/day hydrocortisone) to prevent adrenal crisis. Those taking fludrocortisone were also expected to not change their dose during the study. Participants in Cohort A received oral tildacerfont 200 mg once daily (QD) for 2 weeks, escalating to 600 mg QD for 2 weeks, and then 1000 mg QD for 2 weeks with no washout between doses (Fig. 1). Tildacerfont doses were taken at bedtime or 10 PM. Participants in Cohorts B and C received tildacerfont 200 mg twice daily (BID) or 100 mg BID, respectively, for 2 weeks with dosing at 10 AM and 10 PM. Since absorption of tildacerfont is improved by intake with food, confirmed from a Phase 1 study (16), all doses of tildacerfont were taken within 5 to 15 minutes of eating a standardized snack, composed of approximately 500 calories with 330 calories (37 grams) of fat.

Serial blood samples for biomarker profiles were collected during overnight visits at baseline (days –1/1) and week 2 (days 14/15) of each treatment period. For Cohort A, baseline assessments were conducted from 10 PM to 8

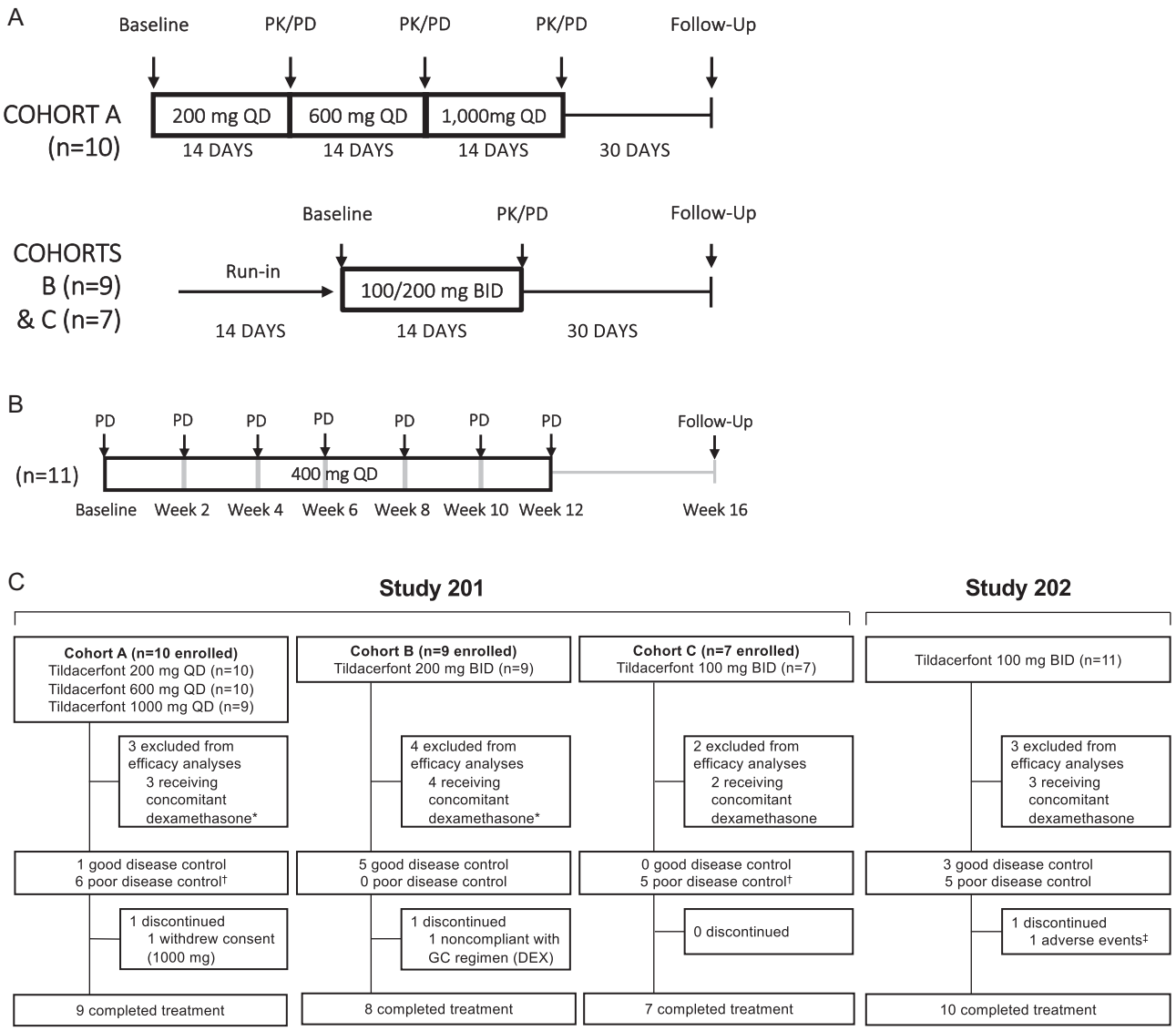

Figure 1. Study designs and patient flow. (A) Study 201 design. Study 201 was a Phase 2a, multicenter, open-label, multiple-dose, dose-escalation study. Participants in Cohort A received oral tildacerfont 200 mg once daily (QD) for 2 weeks, escalating to 600 mg QD for 2 weeks, and then 1000 mg QD for 2 weeks with no washout between doses. Participants in Cohorts B and C received tildacerfont 200 mg twice daily (BID) or 100 mg BID, respectively, for 2 weeks with dosing at 10 AM and 10 PM. Serial blood samples for pharmacodynamic profiles and pharmacokinetic analyses were collected during overnight visits at baseline (days –1/1) and week 2 (days 14/15) of each treatment period. The study enrolled 24 participants overall, but 2 participants contributed data to 2 cohorts. (B) Study 202 design. Study 202 was a 12-week, Phase 2, multicenter, open-label study evaluating oral tildacerfont 400 mg QD. Trial visits were conducted in the morning, approximately 8 AM, prior to consumption of a morning GC dose at baseline (day 1); weeks 2, 4, 6, 8, 10, and 12; and 30 days after the last dose. (C) Patient flow. Poor disease control was defined as androstenedione (A4) > 2× ULN at baseline. Good disease control was defined as A4 ≤ 2× ULN at baseline. *In Study 201, 1 individual on dexamethasone participated in both Cohorts A and B. †In Study 201, 1 individual with poor disease control at baseline participated in both Cohorts A and C. ‡One individual who participated in both studies had liver enzyme elevations during Study 201 and then discontinued Study 202 after experiencing rash without pruritus and smaller increases in liver enzymes.

AM on the night (day –1) and morning (day 1) prior to first dose (day 1 at 10 PM) and over the same time period on days 14 and 15 following the day 14 dose of tildacerfont. Time points for both visits were at 10 PM, and 2, 3, 4, 6, and 8 AM. For Cohorts B and C, baseline assessments were collected from 10 AM (day –1) to 10 AM (day 1), with first dose at 10 AM on day 1, and over the same period on days 14 and 15 with time points at 10 AM and 12, 1, 2, 3, 4, 5, 6, 8, and 10 PM and then at 2, 3, 4, 6, 8 and 10 AM. The primary focus for efficacy assessment was on the 8 AM timepoint corresponding to peak hormone and androgen production prior to consumption of a typical morning GC dose. A follow-up visit was conducted 30 days post last dose for all cohorts.

Study 202

Study 202 was a 12-week, Phase 2, multicenter, open-label study evaluating oral tildacerfont 400 mg QD. As with Study 201, participants remained on a stable GC regimen throughout the study. Doses of tildacerfont were taken at bedtime or 10 PM. Trial visits were conducted in the morning, at approximately 8 AM, prior to consumption of a morning GC dose at baseline (day 1); weeks 2, 4, 6, 8, 10, and 12; and 30 days after the last dose. Visits at weeks 2, 6, and 10 could be conducted as at-home visits, and visits at weeks 4, 8, and 12 were exclusively conducted as in-clinic visits.

In both studies, a safety review committee monitored the available safety, pharmacokinetic, and pharmacodynamic data. For both studies, the GC dose was noted in hydrocortisone equivalents (1 mg prednisolone/prednisone/methylprednisolone = 5 mg hydrocortisone; 1 mg dexamethasone = 80 mg hydrocortisone).

Post Hoc Analyses

Tildacerfont previously demonstrated a moderate drug-drug interaction with midazolam, a drug metabolized by CYP3A4, in a Phase 1 study (16). As dexamethasone is also primarily metabolized by CYP3A4, a post hoc exposure analysis of Study 201 data was performed to investigate the potential drug-drug interaction and its clinical significance in the 21OHD population.

According to the eligibility criteria, all enrolled participants had elevated 17-OHP (≥800 ng/dL; ≥4× ULN) at screening to monitor reductions of at least 1 biomarker. This inclusion criterion enrolled a population with heterogeneous baseline levels of ACTH and A4. Several participants, including the majority of participants in Cohort B, had variably elevated 17-OHP, but their ACTH and A4 levels were near or well within the respective normal ranges. After examining the distribution of data, it was determined that A4 values could be used to separate participants into 2 well-delineated subgroups using a 2× ULN threshold. Using this A4 threshold also resulted in well-segregated baseline ACTH levels within each subgroup at 2× ULN. In contrast, a clear threshold was not identified for 17-OHP, with substantial overlap between the 2 subgroups. The use of baseline A4 to define subgroups is consistent with A4 as the best accepted biomarker of disease control, which is used to guide GC dose titration for adults (2,17). In addition, ACTH represents the most immediate biomarker of CRF1 antagonism (18). We define these 2 subgroups with A4 (and ACTH) > 2× ULN or ≤2× ULN as having baseline "poor disease control" and "good disease control," respectively. In participants classified with good disease control, baseline ACTH and A4 were commonly (80%) within the normal ranges. Efficacy analyses are presented using these subgroups.

Statistical Analyses

For Study 201, a sample size of approximately 6 to 9 participants per cohort was expected to provide sufficient data for an initial estimate of safety and efficacy while maintaining feasible participant recruitment goals for this rare disease. No power calculations were performed. For Study 202, the sample size was based on the number of participants expected to enroll after completing Study 201 or as new participants.

Safety analyses were performed using the safety population, which included all participants who received at least 1 dose of the study drug. Efficacy analyses used the efficacy population, which included all participants who were in the safety population and had both baseline and post-baseline biomarker assessments at 8 AM. The participants included in the pharmacokinetic analysis population were all participants with an evaluable pharmacokinetic profile. As these studies were not powered to detect statistically significant differences, all statistics are descriptive in nature. Geometric means and geometric mean ratios are used to summarize the change over time in biomarkers due to the nonnormality of the data and the large dynamic range of these biomarkers. No imputation of missing data was conducted in Study 201 due to the diurnal pattern of the biomarkers. For participants with missing biomarker data in Study 201, a last observation carried forward approach was used, as all assessments were at approximately the same time across visits. *P*-values were derived to compare good and poor disease control groups for age and body mass index (BMI) from 2-sample *t*-tests assuming equal variance; for comparison of sex and race, from Fisher's exact test; and for GC dose and ACTH, 17-OHP, and A4 levels, from Wilcoxon's signed rank test without continuity correction.

Results

Participant Populations

A total of 24 participants were enrolled in Study 201, with 2 participants participating in 2 dose cohorts (Fig. 1). Study 202 enrolled 11 participants, including 9 who participated in Study 201 and 2 who were treatment naïve. In Study 201, 22 of 24 participants completed the study. One participant in Cohort B was withdrawn by the sponsor due to noncompliance with their GC regimen prior to the day 14 assessments, and 1 participant withdrew consent due to work-related scheduling issues in

Cohort A before initiating the 1000 mg treatment period. In Study 202, 10 of 11 participants completed the study, with 1 participant withdrawn due to an AE, which is summarized in the safety results.

A post hoc drug-drug interaction analysis using Study 201 data demonstrated an approximate 2-fold increase in dexamethasone exposures from baseline to week 2. This increase was considered to be clinically relevant with the potential to confound efficacy assessments. As such, dexamethasone participants [8 participants from Study 201 (1 participant participated in Cohorts A and B) and 3 from Study 202] were excluded from subsequent efficacy analyses but were included in the safety and pharmacokinetic analyses. In Study 201, of the 16 evaluable participants who did not receive dexamethasone therapy, 11 participants were in the poor disease control subgroup. Within cohorts, 6/7 from Cohort A, 0/5 from Cohort B, and 5/5 from Cohort C were in poor disease control, including 1 individual with poor disease control who participated in both Cohorts A and C. In Study 202, 8 of 11 participants did not receive dexamethasone, with 5/8 being classified as having poor disease control at baseline.

Two evaluable participants had partial data that were excluded from the efficacy analyses. For 1 participant in Cohort A, the 200 mg assessments for all 3 biomarkers were excluded due to an intercurrent illness not addressed with stress dosing. For another participant in Cohort A, ACTH data were excluded due to ACTH values were above the upper limit of quantification (2000 pg/mL) at all 8 AM assessments, including baseline, which precluded quantification of change from baseline.

Participant Characteristics

In the evaluable populations of both studies 201 and 202, the mean participant age was 45 years (range 19-67 years) (Table 1). Participants were mildly obese (mean BMI 31.5kg/m^2). The majority of participants reported white as their race with 1 participant reporting multiple races (Asian and white). Eleven (65%) participants in Study 201 and 5 (63%) in Study 202 were female. All female participants were diagnosed with 21OHD within the first year of life, and all male subjects were diagnosed within the first 7 years of life. Fludrocortisone was used in 71% and 100% of the evaluable participants in Studies 201 and 202, respectively. The participants classified as good disease control comprised a higher proportion of female participants than the group classified with poor disease control, 83% *vs* 55% and 100% *vs* 40% for Studies 201 and 202, respectively. The mean total daily dose of background GC in hydrocortisone equivalents was noticeably different between participants classified as poor *vs* good disease control (Study 201: 24.6 *vs* 36.3mg; Study 202: 24.5 *vs* 36.7mg, respectively). The majority of females in Study 202 presented with regular menses at baseline, precluding the ability to characterize improvements in menstrual cyclicity or duration on treatment.

Pharmacokinetics

Pharmacokinetic profiles from Study 201 demonstrated that increasing dose resulted in increasing exposure and close to dose linearity across the dose range studied. No demographic covariates (eg, weight or BMI) were identified to predict exposure levels. Peak exposure levels were observed at approximately 5 to 6 h post dose, reflecting a slow absorption profile (Fig. 2). Representative results from the post hoc drug-drug interaction analysis for 2 participants are presented in Figure 3, demonstrating that administration of tildacerfont resulted in higher exposures of dexamethasone. On average, this increase in dexamethasone exposure was approximately 2-fold using an area under the curve metric. This increased exposure was deemed to be potentially clinically relevant, resulting in the exclusion of these participants from efficacy analyses.

Efficacy

Study 201

After receiving tildacerfont for 14 days, the levels of ACTH, 17-OHP, and A4 were reduced throughout the expected diurnal rise in the early morning period in participants classified with poor disease control, with notable reductions observed at the 8 AM time point (Table 2; Fig. 4). No dose-response relationship was observed across the evaluated dose range despite increasing tildacerfont exposure with dose, and no additional benefit was observed when tildacerfont total daily dose of 200 mg was dosed as 200 mg QD or 100 mg BID. For participants classified with good disease control, mean baseline levels of ACTH and A4 were below the upper limit of normal, and treatment with tildacerfont did not further reduce these levels in a clinically relevant manner as the biomarker changes in this subgroup were numerically small (Table 2). Among participants in the good disease control cohort who had elevated levels of 17-OHP, tildacerfont led to a modest decrease in 17-OHP.

Study 202

Among participants with poor disease control at baseline, reductions in ACTH were observed by week 2 of receiving 400 mg QD that were numerically similar to the observed reductions when receiving 200 mg QD in Study 201 (58% *vs* 47% in Study 202 and Study 201, respectively) (Fig. 5, Table 2). The reductions in ACTH at week 2 corresponded

Table 1. Demographics and baseline characteristics of nondexamethasone participants

	Study 201			Study 202		
	Good Disease Control (N = 6)	Poor Disease Control (N = 11)	*P*-value	Good Disease Control (N = 3)	Poor Disease Control (N = 5)	*P*-value
Age (year), mean (SD) [range]	44 (16.6) [19,66]	45 (17.0) [19, 67]	0.8538[a]	48(17.7) [32, 67]	42 (15.6) [26, 63]	0.6556[a]
Sex, female, n (%)	5 (83)	6 (55)	0.3340[b]	3 (100)	2 (40)	0.1964[b]
Race, white, n (%)	6 (100)	10 (91)	1.000[b]	3 (100)	4 (80)	1.000[b]
BMI (kg/m^2), mean (SD)	31.3 (5.8)	29.9 (5.9)	0.6482[a]	35.5 (6.1)	27.8 (5.6)	0.1148[a]
Glucocorticoid dose (HC emg), mean (SD)	36.3 (8.0)	24.6 (8.6)	0.0141[c]	36.7 (11.6)	24.5 (11.5)	0.2662[c]
Glucocorticoid type			0.8100[b]			0.6786[b]
Hydrocortisone, n (%)	2 (33)	6 (55)		0	2 (40)	
Pred family, n (%)	3 (50)	4 (36)		2 (67)	1 (20)	
Combination, n (%)	1 (17)	1 (9)		1 (33)	2 (40)	
Fludrocortisone use, n (%)	5 (83)	7 (64)	0.6000[b]	3 (100)	5 (100)	1.000[b]
ACTH (pg/mL) 8 AM, geometric mean (CV%)	30.9 (273)	397.0 (89)	0.0011[c]	12.2 (584)	536.6 (109)	0.0253[c]
17-OHP (ng/dL) 8 AM, geometric mean (CV%)	1531.6 (489)	6688.6 (113)	0.0270[c]	314.1 (1069)	15 323.3 (47)	0.0253[c]
A4 (ng/dL) 8 AM, geometric mean (CV%)	97.6 (338)	333.1 (171)	0.1594[c]	28.8 (216)	1001.1 (48)	0.0253[c]

Adrenocorticotropic hormone (ACTH) reference range: 10-63.3 pg/mL; 17-hydroxyprogesterone (17-OHP) reference range: 27-199 ng/dL; androstenedione (A4) reference range: males 27-151 ng/dL, premenopausal females: 41-262 ng/dL, postmenopausal females: 17-99 ng/dL; hydrocortisone equivalent (HCe): 1 mg prednisolone/prednisone/methylprednisolone (Pred) = 5 mg hydrocortisone. Combination therapy: combination of hydrocortisone and a member of the pred family. Participants with poor disease control had baseline ACTH and A4 levels that were more than twice the upper limit of normal. Participants with good disease control had baseline acth and ACTH and A4 that were less than twice the upper limit of normal.

[a]*P*-value was calculated from two-sample t-test assuming equal variance.

[b]*P*-value was calculated from Fisher's exact test.

[c]*P*-value was calculated from Wilcoxon's signed rank test without continuity correction.

Abbreviations: BMI, body mass index; CV, coefficient of variation; SD, standard deviation.

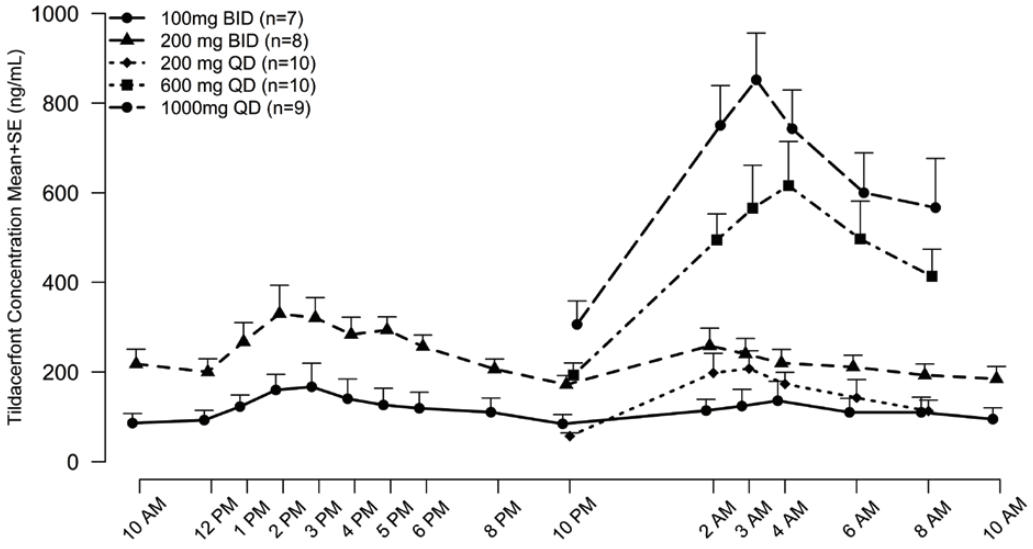

Figure 2. Tildacerfont pharmacokinetics profiles (Study 201). In Cohort A [200, 600, or 1000 mg once daily (QD)], participants received tildacerfont at 10 PM. One participant in Cohort B and 1 participant in Cohort A at 1000 mg were discontinued prior to day 14 pharmacokinetic assessments. Participants who received twice daily (BID) dosing of tildacerfont received the dose at 10 AM and 10 PM each day.

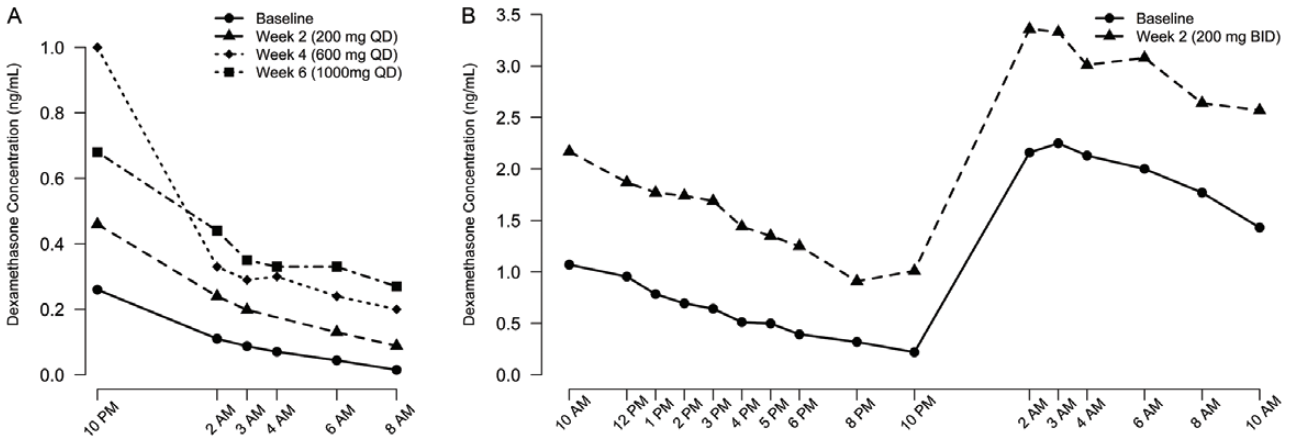

Figure 3. Representative drug-drug interaction between tildacerfont and dexamethasone (n = 1; Study 201). Representative participants demonstrating increase in dexamethasone exposures with administration of tildacerfont. (A) Tildacerfont dose-dependent increase in dexamethasone exposures is observed in the participant in Cohort A receiving doses from 200 to 1000 mg once daily (QD) with dexamethasone dosing prior to time 0. (B) Increase in dexamethasone exposure with tildacerfont 200 mg twice daily (BID) over 24 h with administration of dexamethasone at approximately 10 PM (12 h post AM dose).

with smaller magnitude reductions in 17-OHP and A4, also similar to the Study 201 results at 200 mg QD. After week 2, further reductions in all biomarkers were observed, with maximum mean reductions of approximately 80% in ACTH, 17-OHP, and A4 and mean biomarker levels reaching near target by week 12 (Fig. 5). At the mean level, the majority of the total reduction in ACTH was observed by week 2, while the majority of the total reductions in 17-OHP and A4 were observed post week 2 through week 12.

The biomarker reductions across the 12-week period were observed for 4 of 5 participants with poor disease control, including normalization of ACTH in 3 of 5 participants (60%), 1 participant by week 2 and 2 participants during weeks 10 and/or 12. Furthermore, 2 of 5 participants (40%) achieved normalization of A4 at weeks 10 and/or 12. After treatment discontinuation, biomarker levels regressed back toward baseline levels. One male participant (aged 54 years) in the poor disease subgroup who participated in both studies did not respond to treatment based on ACTH, 17-OHP, and A4 responses.

Among participants with good disease control at baseline, changes in ACTH, 17-OHP, and A4 were small and were relatively flat throughout the 12-week treatment period (Fig. 5). Tildacerfont did not appear to further suppress ACTH, 17-OHP, or A4 levels.

Table 2. Summary of change in biomarkers from baseline to week 2 at 8 AM in Study 201 in participants not taking concomitant dexamethasone therapy

	Poor Disease Control				Good Disease Control	
	Cohort A			Cohort C	Cohort A	Cohort B
	200 mg QD n = 6	600 mg QD n = 6	1000 mg QD n = 6	100 mg BID n = 5	200 mg QD n = 1	200 mg QD n = 5
ACTH						
Baseline, GM (CV%) [n]	501.3 (105.3) [4]	473.1 (88.3) [5]	473.1 (88.3) [5]	333.2 (95.5) [5]	112.7 (NA) [1]	23.89 (278.2) [5]
Week 2, GM (CV%)	266.2 (43.8)	192.2 (62.6)	444.4 (113.8)	238.6 (298.8)	187.4 (NA)	22.98 (232.4)
% change (95% CI)	−46.9 (−85.2, 91.1)	−59.4 (−83.9, 2.3)	−6.1 (−29.9, 25.8)	−28.4 (−76.0, 114.1)	66.3 (NA)	−3.8 (−36.7, 46.3)
17-OHP						
Baseline, GM (CV%) [n]	6629.5 (154.4) [5]	8910.5 (96.9) [5]	6778.5 (128.8) [6]	6582.2 (113.5) [5]	11249.0 (NA) [1]	2137.1 (46.3) [4]
Week 2, GM (CV%)	5545.2 (211.8)	8483.9 (188.8)	6800.1 (248.7)	4061.3 (188.2)	9786.0 (NA)	1266.2 (81.8)
% change (95% CI)	−16.4 (−42.2, 21.1)	−4.8 (−50.2, 81.9)	0.3 (−44.1, 80.1)	−38.3 (−77.4, 68.5)	−13.0 (NA)	−40.8 (−83.5, 112.9)
A4						
Baseline, GM (CV%) [n]	405.6 (234.9) [5]	425.4 (188.2) [6]	425.4 (188.2) [6]	248.3 (167.2) [5]	533.0 (NA) [1]	69.5 (296.9) [5]
Week 2, GM (CV%)	332.2 (184.8)	322.7 (296.6)	332.5 (378.0)	193.6 (180.8)	499.0 (NA)	76.5 (216.6)
% change (95% CI)	−18.1 (−42.1, 15.9)	−24.2 (−50.5, 16.2)	−21.8 (−56.0, 38.9)	−22.0 (−53.5, 31.0)	−6.4 (NA)	10.0 (−59.2, 196.3)

No participant in Cohort B qualified as having poor disease control. Participants with poor disease control had baseline adrenocorticotropic hormone (ACTH) and androstenedione (A4) levels that were more than twice the upper limit of normal. Participants with good disease control had baseline ACTH and A4 that were less than twice the upper limit of normal.

Abbreviations: 17-OHP, 17-hydroxyprogesterone; BID, twice daily; CV, coefficient of variation; GM, geometric mean; QD, once daily.

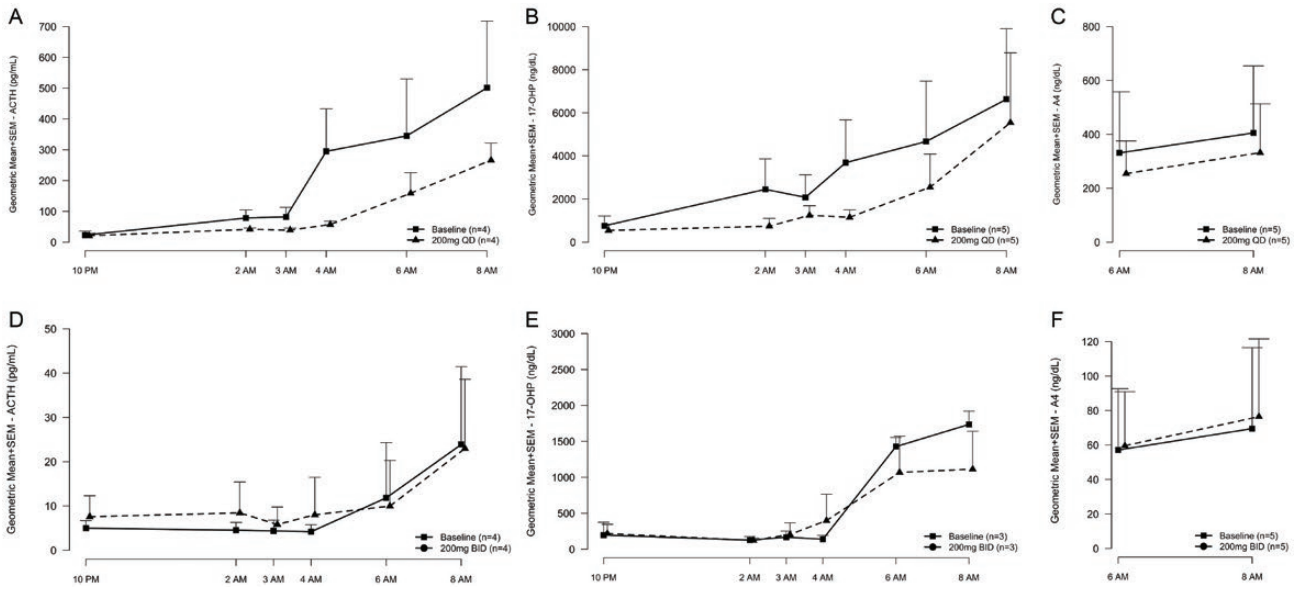

Figure 4. Overnight serum adrenocorticotropic hormone (ACTH), 17-hydroxyprogesterone (17-OHP), and androstenedione (A4) levels at baseline and after 2 weeks of tildacerfont (Study 201). No participants at 200 mg twice daily (BID) qualified as having poor disease control. Participants with missing data (either baseline or 8 AM) are not included. Participants with poor disease control had baseline ACTH and A4 levels that were more than twice the upper limit of normal. Participants with good disease control had baseline ACTH and A4 levels that were less than twice the upper limit of normal. Abbreviation: QD, once daily.

Treatment Effects on TARTs

In Study 201, 3 male participants had detectable TARTs at baseline by scrotal ultrasound, but only 1 participant (hydrocortisone 30 mg daily) from Cohort A participated in an end-of-study scrotal ultrasound (Fig. 6). Baseline testicular ultrasound confirmed the testicles were small

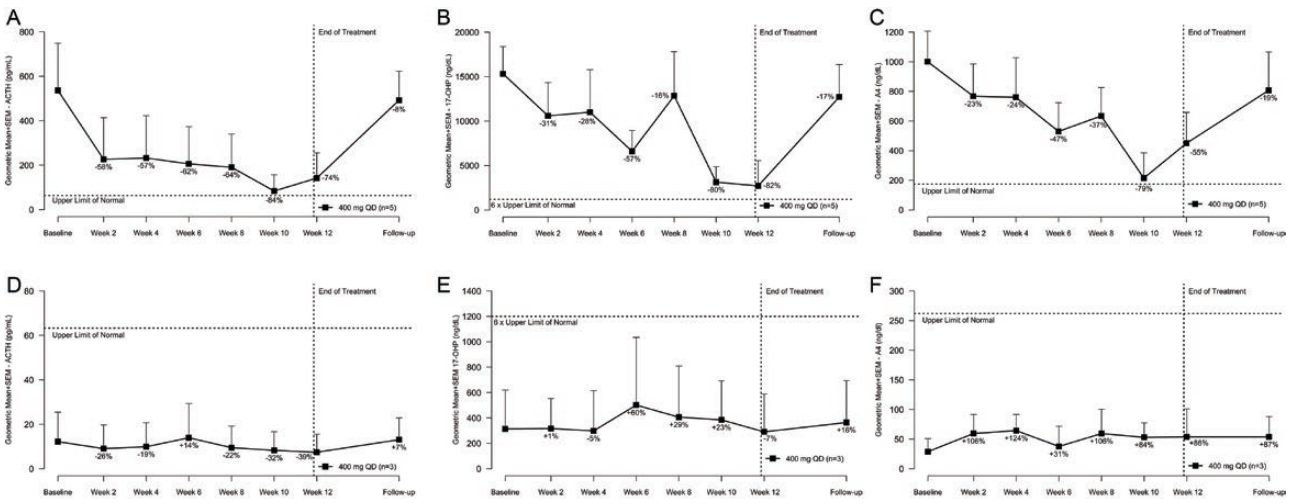

Figure 5. Changes from baseline in adrenocorticotropic hormone (ACTH), 17-hydroxyprogesterone (17-OHP), and androstenedione (A4) levels at 8 AM during 12 weeks of tildacerfont treatment by baseline disease control status (Study 202). Participants were required to be on a stable regimen of glucocorticoid replacement for a minimum of 30 days before baseline that was expected to remain stable throughout the study. Assessments were taken at approximately 8 AM prior to morning glucocorticoid dose. Geometric means are presented with percentage reduction derived from the geometric mean ratios. Axes are different between poor and good disease control to enable presentation of good disease control participants with mean values below the target levels. Abbreviation: QD, once daily.

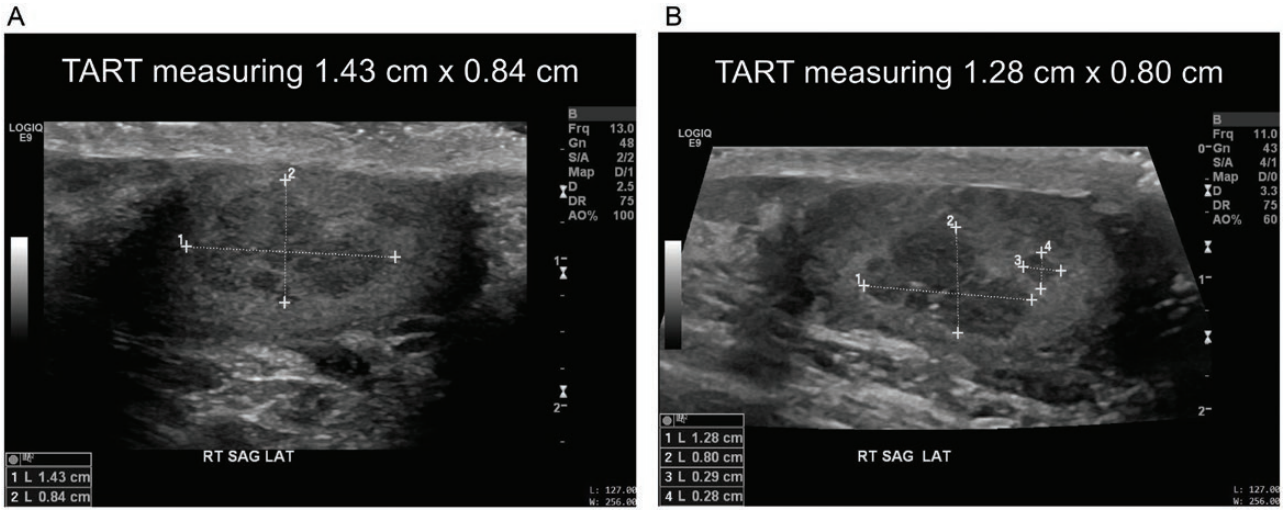

Figure 6. Changes in testicular adrenal rest tumor (TART) measurements in 1 participant in Study 201. Pretreatment and post 6-week tildacerfont treatment testicular ultrasounds of single TART in a participant. Treatment with tildacerfont reduced the TART approximately 23% in volume.

and bilaterally heterogeneous (right: 3.6 × 2.4 × 1.8 cm; left: 3.5 × 3.4 × 1.9 cm), with a hyperechogenic hypervascularized tumor mosaic area of 1.43 × 0.84 cm (volume 1.37 cm^3) and potential for duct occlusion (Fig. 6A). After 6 weeks of treatment, the volume of this participant's TART was reduced 23%, corresponding to reductions in ACTH of 87% from baseline levels after 6 weeks of treatment.

In Study 202, 3 male participants had detectable TARTs at baseline but only 2 of these participants had a posttreatment ultrasound. One participant, the nonresponder in the poor disease subgroup, had no change observed in TART volume, which corresponds to the observed lack of change in ACTH levels. The second participant had 4 detectable TARTs at baseline and no detectable TARTs post treatment. This participant, classified as poor disease control, was on dexamethasone (0.375 mg) and the assignment of efficacy is confounded by the increase in dexamethasone exposures.

Safety

The most frequent treatment-emergent AEs are described in Table 3. The majority of AEs in both studies were

Table 3. Summary of treatment-emergent adverse events in 2 or more participants across Studies 201 and 202 (safety population)

Preferred term	Study 201					Study 202	
	Cohort A			Cohort B	Cohort C		
	200 mg QD (n = 10)	600 mg QD (n = 10)	1000 mg QD (n = 9)	200 mg BID (n = 9)	100 mg BID (n = 7)	400 mg QD (n = 11)	Total (N = 56)
Participants with at least 1 TEAE, n (%)	6 (60.0)	3 (30.0)	3 (33.3)	5 (55.6)	4 (57.1)	9 (81.8)	30 (53.6)
Adverse events occurring in at least 2 participants across both studies, n (%)							
Headache	0	0	1 (11.1)	2 (22.2)	0	1 (9.1)	4 (7.1)
Upper respiratory tract infection	0	2 (20.0)	0	0	0	2 (18.2)	4 (7.1)
Contusion	0	0	0	1 (11.1)	1 (14.3)	1 (9.1)	3 (5.4)
Diarrhea	1 (10.0)	1 (10.0)	0	0	0	1 (9.1)	3 (5.4)
Pruritus	0	0	1 (11.1)	1 (11.1)	0	1 (9.1)	3 (5.4)
Back pain	0	0	1 (11.1)	0	0	1 (9.1)	2 (3.6)
Flatulence	0	1 (10.0)	0	1 (11.1)	0	0	2 (3.6)
Hyperhidrosis	1 (10.0)	0	0	1 (11.1)	0	0	2 (3.6)
Hepatic enzyme increased	0	0	1 (11.1)	0	0	1 (9.1)	2 (3.6)
Nausea	0	0	1 (11.1)	0	0	1 (9.1)	2 (3.6)
Salivary gland enlargement	1 (10.0)	1 (10.0)	0	0	0	0	2 (3.6)

The adverse event of increased hepatic enzyme in Study 201 and Study 202 occurred in the same participant.
Abbreviations: BID, twice daily; QD, once daily; TEAE, treatment-emergent adverse event.

deemed mild (grade 1), judged by investigators to be unrelated to study drug, and occurred as single events. The highest-grade AE was a grade 3 hot flush observed in a female (aged 48 years) participant in Study 201 deemed related to treatment. This AE occurred on days 4 and 5 of the 200-mg treatment period, lasted less than 30 min in each occurrence, and resolved without intervention. No dose pattern was observed for any AE, and no serious AEs or deaths were reported. One subject reported stress dosing for cough, chills, and fatigue on days 4 to 5 in the 100 mg BID cohort in Study 201. No other subjects recorded stress dosing for an intercurrent illness.

In Study 201, on the last day of the tildacerfont 1000-mg dose period, a 25-year-old male experienced transaminase elevations including alanine aminotransferase (ALT; between 5× and 9× ULN) and smaller changes (<5× ULN) in aspartate aminotransferase (AST), without a change in direct bilirubin. The participant had not experienced changes to ALT or AST at the 200-mg and 600-mg QD dose levels. The transaminase elevations resolved within 30 days. Approximately a year post finishing Study 201, the same participant enrolled in Study 202 and experienced pruritus without rash on days 21 and 24, smaller magnitude transaminase elevations, <3× ULN in ALT and <2× ULN in AST, with no change to direct bilirubin observed. Because the participant had also experienced transaminase elevations in Study 201, the participant was discontinued from Study 202 due to these AEs. The transaminase elevations resolved within 15 days without further medical intervention. No other clinically relevant changes in chemistry or hematology parameters were observed.

Discussion

In 2 Phase 2 trials, tildacerfont reduced ACTH, A4, and 17-OHP in participants with 21OHD classified with poor disease control at baseline. The reductions in ACTH observed in Study 201 provided proof of concept for the use of tildacerfont in the treatment of 21OHD, in that the changes in ACTH provided clinical evidence of CRF1 receptor antagonism. Study 201 further showed potential efficacy to reduce key adrenal steroid biomarkers. Reductions in ACTH translated into consistent reductions in A4 across the various dose levels, demonstrating the potential of tildacerfont to improve disease control. While reductions in 17-OHP were inconsistent across doses at single time points, this finding may be reflective of inherent fluctuations in 17-OHP compared to A4 that have been observed after GC dosing in other studies (17). Examining the overnight time courses of ACTH and 17-OHP in Study 201 at 200 mg QD (Fig. 4A and 4B), the week 2 ACTH and 17-OHP levels were consistently reduced across time points, demonstrating an effect when evaluating multiple time points.

These biomarker reductions occurred at all dose levels of tildacerfont studied, with no apparent dose-response

effect. Based on the findings from Study 201, the lowest effective dose of tildacerfont is not yet known, but doses above 200 mg/day are not likely to produce greater efficacy responses. Additionally, BID dosing did not result in added benefit in biomarker reductions, which is consistent with the terminal half-life of tildacerfont (~60 h) (16). The results of a prior Phase 1b study in women with 21OHD treated with single doses of the CRF1 receptor antagonist NBI-77860 support the mechanism of action and current study findings for tildacerfont. In that study, 17-OHP was reduced by up to 27% and ACTH by up to 43% (18). Additionally, the observed reduction in ACTH is a plausible explanation for the reduction in TART volume observed in one participant in Study 201. Although only 1 evaluable participant with a TART had pre- and postdose ultrasounds in that study, the observed reduction in TART volume raises the possibility that improved clinical outcomes might result from sustained reductions in ACTH.

Study 202 results supported and improved upon the Study 201 results. The week 2 biomarker reductions from Study 202 at 400 mg QD in the poor disease control subgroup were consistent with the findings from Study 201 at 200 mg QD, further demonstrating a lack of dose-response at doses of 200 mg QD and above. Study 202 demonstrated that further improvements in biomarker control should be expected with longer term dosing beyond 2 weeks. Participants with poor disease control at baseline in Study 202 demonstrated improved biomarker control, with large biomarker reductions over the 12-week treatment period while remaining on a stable background GC therapy. Several of these participants achieved normalization of ACTH and/or A4. We are not aware of any other investigational product candidates having reported normalization of these highly elevated biomarkers within 12 weeks in participants with 21OHD without requiring increases to the daily GC doses. Additionally, these data suggest that 17-OHP and A4 reductions may lag behind declines in ACTH concentrations for some patients. This pattern also informs us when to monitor for those biomarkers in anticipation of GC dose changes, in long-term studies aimed at reducing GC doses. Despite fluctuations in the biomarkers over time—especially 17-OHP—the results of Study 201 and 202 suggest that long-term treatment with tildacerfont generally induces reductions in ACTH that translate to reduced 17-OHP and A4 in patients with 21OHD and poor disease control.

It was noted that 1 participant in the poor disease control subgroup, who participated in both Study 201 and Study 202, did not respond to treatment. The participant's tildacerfont exposure was within the expected limits. While the participant's ACTH and A4 levels were highly elevated at baseline, at 1030 pg/mL and 1813 ng/dL for ACTH and A4 in Study 202, respectively, another participant in the same study had similar highly elevated baseline levels but demonstrated large reductions across all 3 biomarkers. The only notable difference between the 2 participants was age: the nonresponder was 54 years old, whereas the responder was 26 years old. Future studies will treat a larger number of subjects and for longer duration, which may shed light on why some patients might exhibit a poor treatment response.

In both Study 201 and Study 202, participants with good disease control at baseline generally experienced small biomarker changes, given that the baseline and postbaseline levels were generally well below the upper limit of normal. A key difference between the 2 biomarker control subgroups was the total daily GC dose, with participants in the good disease control subgroup having an approximately 44% higher total daily GC dose, compared to the poor disease subgroup, to achieve biomarker control.

Tildacerfont was generally well tolerated and safe. No new safety signals were observed during either study compared to the safety profile observed in healthy participants, and no new safety concerns were identified with 12-week dosing *vs* 2-week dosing. The majority of AEs were mild in nature and deemed unrelated to treatment. No serious AEs were reported at any dose. Headache was the most common AE along with upper respiratory tract infection, and 1 participant had AEs of transient transaminase elevations, without changes in direct bilirubin, in both studies that resolved without medical intervention.

Tildacerfont is a lipophilic compound that results in poor absorption, in the fasted state, which is enhanced by administration with food. In these studies, tildacerfont was administered with a standardized snack, which might cause weight gain. Small changes were observed in BMI, mean (SD): a reduction in BMI of 0.1 (1.05) kg/m^2 after 6 weeks in Study 201 Cohort A and an increase of 0.3 (1.09) kg/m^2 after 12 weeks in Study 202. Given the lack of a placebo control in these studies, the interpretation of these small changes is not clear. With prolonged tildacerfont therapy, reduced GC doses might mitigate any potential for weight gain from the administration schedule. Ongoing studies have moved dosing from bedtime to correspond with a moderate-fat meal (consistent with a standard American diet) at dinnertime, thus removing the need for a late-night snack. Weight-based endpoints are part of the ongoing longer duration studies with tildacerfont.

The magnitude of response over 12 weeks in Study 202 suggests tildacerfont may have the potential to, first, allow participants with 21OHD and poor disease control to achieve improved disease control without increasing the total daily GC dose and, second, for those with good disease control, the potential to reduce their total daily GC

dose over time to levels similar to the poor disease control subgroup while maintaining biomarker control. Longer-term studies are needed to better understand the potential of these treatment effects over time, and two 52-week studies (NCT04457336, NCT04544410) are ongoing to address this question in the 2 subgroups separately. The first study will further evaluate the potential of tildacerfont to normalize biomarkers in participants with poor disease control without changing GC dose, and the second study will evaluate the GC-sparing potential of tildacerfont in participants with good disease control along with improvements in clinical outcomes in both studies.

This study had several limitations. The number of participants (mostly white) in these studies was small and represented a heterogeneous population including participants as young as 19 and as old as 67 years of age, lean and obese participants, and participants with varying levels of baseline ACTH and adrenal steroid biomarkers. A drug-drug interaction with dexamethasone posed another study limitation. Whereas no drug-drug interactions were observed between hydrocortisone or predniso(lo)ne and tildacerfont, an interaction was identified between dexamethasone and tildacerfont in post hoc analyses that led to a decision to exclude from the efficacy analyses participants who received concomitant dexamethasone. Although this data censoring was done to avoid confounding study results from the drug-drug interaction, the exclusion of these participants was a study limitation that further reduced an already small sample size and may have influenced the efficacy results. This consideration is important, as dexamethasone is a common treatment used in adults with 21OHD. Since one long-term goal of tildacerfont treatment would be to decrease supraphysiologic GC exposure, switching participants from long-acting dexamethasone to short-acting hydrocortisone for concurrent treatment with tildacerfont may be beneficial for chronic management. Additional limitations are that these studies were not designed to assess other key outcome measures such as clinical endpoints or the ability to control disease while reducing GC dosing. Future studies with a goal of identifying factors that predict response to tildacerfont may support treatment decision-making and reduce the time spent optimizing GC dose, which can be a lengthy process.

In conclusion, efficacy data from these open label studies demonstrated proof of concept through the achievement of receptor engagement, indicated via reductions in ACTH and in 17-OHP and A4, the latter of which are adrenal steroid biomarkers of 21OHD. In Study 201, the mean levels of ACTH, 17-OHP, and A4 were reduced relative to baseline across all doses tested. Study 202 showed that continued reductions could be achieved across each of the key biomarkers with longer term therapy, including normalization of ACTH and A4 levels in several participants who had significantly elevated levels at baseline. Tildacerfont was generally well-tolerated and safe. The findings from these studies support the ongoing late-stage studies of tildacerfont in participants with 21OHD.

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Additional Information

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Data Availability: Data sets generated during and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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