Official Title: A Phase IV, Multicenter, Open-Label Study of the Immunogenicity of Nutropin AQ® V1.1 [Somatropin (rDNA Origin) Injection] Administered Daily to Naïve Growth Hormone-Deficient Children (iSTUDY)

NCT Number: NCT02311894

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STATISTICAL ANALYSIS PLAN

TITLE: **A PHASE IV, MULTICENTER, OPEN-LABEL STUDY OF THE IMMUNOGENICITY OF NUTROPIN AQ® V1.1 [SOMATROPIN (rDNA ORIGIN) INJECTION] ADMINISTERED DAILY TO NAÏVE GROWTH HORMONE-DEFICIENT CHILDREN** *(iSTUDY)*

PROTOCOL NUMBER: ML29543

STUDY DRUG: Nutropin AQ® (Somatropin [rDNA origin] injection)

VERSION NUMBER: 1.4

IND NUMBER: BB IND 39,305

EUDRACT NUMBER: Not applicable

SPONSOR: Genentech, Inc.

PLAN PREPARED BY: [redacted]

DATE FINAL: See electronic date stamp below.

STATISTICAL ANALYSIS PLAN APPROVAL

Statistical Analysis Plan (SAP) Final Sign-Off Sheet

Study title:	**A PHASE IV, MULTICENTER, OPEN-LABEL STUDY OF THE IMMUNOGENICITY OF NUTROPIN AQ® V1.1 [SOMATROPIN (rDNA ORIGIN) INJECTION] ADMINISTERED DAILY TO NAÏVE GROWTH HORMONE-DEFICIENT CHILDREN *(iSTUDY)***		
Protocol #:	**ML29543**	**DAP Version: 1.4**	**Final**

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* The Biostatistics approver has ensured that key team members have been involved, contributed and reviewed the content of the List of Planned Outputs as described in the SAP Module guideline.

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1. BACKGROUND

Nutropin [somatropin (rDNA origin) for injection] is a lyophilized form of human growth hormone (hGH) produced by recombinant DNA technology. Nutropin AQ is a liquid formulation with the chemical structure of somatropin identical to lyophilized Nutropin.

To use the most advanced methodology and equipment to ensure a continued supply of Nutropin AQ, Genentech has updated its manufacturing process while continuing to use the same *Escherichia coli* growth hormone production system. Chemical and biological characterization (including potency) has demonstrated comparability of drug substance produced by the original and updated manufacturing processes and, based on this, the new manufacturing process that is used to produce Nutropin AQ v1.1 has been approved by FDA.

This study, performed in children with growth hormone deficiency (GHD), will assess the safety and immunogenicity profile of Nutropin AQ produced by the new manufacturing process.

For further background information please see Section 1 of the protocol.

This Statistical Analysis Plan (SAP) describes the statistical methods to be used for study ML29543. This version of the SAP has been developed using version 2 of the study protocol dated 22 April 2015 and revision 1.2 of the CRF dated 26 July 2017.

2. STUDY DESIGN

This is a Phase IV, multicenter, open-label, single-arm study of somatropin (rDNA origin) (Nutropin AQ v1.1) in naïve prepubertal children with GHD. Approximately 80 patients will be enrolled at approximately 30 sites in the U.S. and will be treated with daily subcutaneous (SC) injections of Nutropin AQ v1.1 at a dose of up to 0.043 mg/kg/day (0.3 mg/kg/week, which is the approved and indicated dosage for pediatric GHD; refer to the U.S. Prescribing Information [USPI] for Nutropin AQ) for 12 months. Dose adjustments may be made at the Month 6 visit for changes in weight and insulin growth factor-1 (IGF-1) levels, if measured.

2.1 PROTOCOL SYNOPSIS

The Protocol Synopsis is in Appendix 1. For additional details, see the Schedule of Assessments in Appendix 2.

2.2 OUTCOME MEASURES

2.2.1 Primary Outcome Measures

- The primary outcome measure for this study is the occurrence of anti-GH antibodies in rhGH-naïve, prepubertal GHD children treated with Nutropin AQ v1.1.

2.2.2 Secondary Outcome Measures

- The occurrence of functional growth attenuation in association with positive antibody formation in patients treated with Nutropin AQ v1.1

  This will be considered in any patient who demonstrates an initial growth response greater than pretreatment velocity after treatment with Nutropin AQ v1.1, but then slows to below pretreatment velocity in the ensuing 6- to 12-month period (or reaches ≤2 cm per year)

- The difference between the annualized 6- and 12-month growth velocities for patients who were anti-GH antibody-positive at any post-baseline visit and those who were anti-GH antibody-negative at all visits (including baseline)

- The formation of neutralizing antibodies, and the association between the presence of neutralizing anti-GH antibodies and growth attenuation.

2.2.3 Exploratory Efficacy Outcome Measures

This section is not applicable.

2.2.4 Pharmacokinetic Efficacy Outcome Measures

This section is not applicable.

2.2.5 Safety Outcome Measures

No specific safety assessments are planned for this study. However, analysis of all safety events will be performed.

2.3 DETERMINATION OF SAMPLE SIZE

A target enrollment of 80 patients was chosen to allow for a projected 10–15% dropout rate and exclusion of growth data for an estimated 10% of patients who might enter puberty during the study period and leave sufficient patient numbers to compare to previous studies of immunogenicity. This target enrollment was based on enrollment in a previous study (L0368) which planned to enroll 60 patients for the assessment of growth rate and change in height standard deviation score (SDS). An initial sample size was chosen to ensure that at least 50 patients would complete the study (L0368 FR 30SEP97; Sec 4.8.4).

This study is expected to yield a minimum of 50 evaluable patients. Given a sample size of 50, and an assumed 50% of patients developing anti-GH antibodies, the 95% confidence interval (CI) around this proportion will be (35.53%, 64.47%). This interval was calculated using Clopper-Pearson methodology. Fluctuations in the proportion of patients developing anti-GH antibodies and the increase of evaluable patients will result in more precise confidence intervals. The exact confidence intervals for the scenarios for sample size of 50 and 60 in combination with the proportion of patients who are anti-GH antibody positive of 50% and 20% are listed in Table 1.

Table 1

Clopper-Pearson 95% Confidence Intervals for the Proportion of Patients Who Are Anti-GH Antibody Positive Based on 50 and 60 Patients

N	Proportion of Patients Who Are Anti-GH Antibody Positive	95% Confidence Interval (Lower Limit)	95% Confidence Interval (Upper Limit)
50	0.5	0.3553	0.6447
60	0.5	0.3681	0.6319
50	0.2	0.1003	0.3372
60	0.2	0.1078	0.3230

2.4 ANALYSIS TIMING

No interim analysis is planned. The final analysis will be performed after the completion of the study and after the clinical database is locked.

3. STUDY CONDUCT

3.1 RANDOMIZATION ISSUES

There is no randomization for this open-label, single arm study.

3.2 INDEPENDENT REVIEW FACILITY

This section is not applicable.

3.3 DATA MONITORING

There is no independent data monitoring committee planned for this study.

4. STATISTICAL METHODS

Data listings will be produced for all endpoints. Descriptive statistics and graphic displays will be provided to summarize data from this study. There are no formal statistical hypothesis tests planned.

Categorical variables will be summarized as the number and percentage of patients or occurrences in each response category. Continuous variables will be summarized using descriptive statistics (number of observations, mean, standard deviation, median, minimum and maximum).

4.1 ANALYSIS POPULATIONS

There are three analysis populations for this study: Intent-to-treat (ITT), Safety and Modified ITT.

4.1.1 Intent-to-Treat (ITT) Population

The ITT Population includes all patients enrolled in the study.

4.1.2 Per Protocol Population

This section is not applicable.

4.1.3 Pharmacokinetic-Evaluable Population

This section is not applicable.

4.1.4 Safety Population

The Safety Population includes all enrolled patients (i.e. ITT population) who received at least one dose of study drug.

4.1.5 Modified Intent-to-Treat (mITT) Population

The Modified ITT Population includes all enrolled patients (i.e. ITT population) who received at least one dose of study drug and had at least one post-baseline assessment (e.g., lab, vital signs, physical exam, concomitant medication, and adverse event).

4.2 ANALYSIS OF STUDY CONDUCT

The number and percentage of patients who have signed informed consent, met eligibility criteria, had major protocol deviations, and received treatment will be tabulated. Reasons for not completing the study will also be tabulated using numbers and percentages.

A summary of the duration of the patient participation in the study will be produced. Duration will be calculated as the date of the last study assessment minus the first study drug administration date + 1.

The number and percentage of patients included and excluded from the analysis populations will be tabulated. Reason(s) for exclusion from each population will be similarly summarized.

4.3 ANALYSIS OF TREATMENT GROUP COMPARABILITY

This is a single-arm study therefore only descriptive statistics will be provided; no treatment group comparison will be performed.

Clinically important demographic and disease characteristics at baseline will be summarized overall. Summaries will be produced for the ITT population.

Continuous variables will be summarized by the number of non-missing observations, mean, standard deviation, median, minimum and maximum values. Categorical variables will be summarized using numbers and percentages of patients in each category; unknown or missing will be included as a separate category.

The variables to be summarized include but are not limited to the following:

- Demographics: sex, ethnicity, race(s), age (continuous).

- Baseline characteristics: height, weight, height standard deviation score (SDS) (continuous and categorized as ≤ -3, >-3 to ≤ -2, >-2 to ≤ -1, >-1), pre-treatment growth velocity, and prepubertal Tanner stage < 1 (yes or no). See Appendix 3 for the SAS macros for the calculation of normal height SDS (z-scores) for this study.

  Pre-treatment growth velocity is calculated from the baseline and last historical height measurements as: 365.25 × (Baseline height – last historical height)/(date of baseline height – date of last historical height)
- Disease-related characteristics: birth weight, bone age, diagnosis of Turner syndrome.
- Baseline antibody testing (anti-growth hormone antibody screening assay (ATA), anti-growth hormone neutralizing antibody assay (Nab) and anti-growth hormone antibody binding capacity assay (ABC).
- Medical history in specific categories.
- Historical Laboratory Results on GH stimulation test (maximum GH level, insulin level, L-Dopa level), thyroid function test (free T4, total T4, TSH) in 12 months prior to informed consent/assent.
- Screening/Baseline physical examination results, including presence of hip or knee pain, fundoscopic examination and appearance of spine.

Subject listings on demographics, baseline characteristics, medical history, screening physical examination and historical laboratory measurements will be provided.

4.4 OUTCOME ANALYSES

Outcome analyses will be performed on the modified ITT population. In the analyses related to growth attenuation, growth velocity and height SDS, patients who enter puberty during the study period will be included only up to the last visit at which they were determined to be prepubertal.

The study windows listed below in Table 2 (based on visit dates) will be used:

Table 2 Study Windows

Visit	Target Day	Window
Screening/Baseline	Day -28 to -1	Day -28 to 1
Month 1	Day 30	Day 2 to 60
Month 3	Day 90	Day 61 to 135
Month 6	Day 180	Day 136 to 225
Month 9	Day 270	Day 226 to 315
Month 12	Day 360	Day 316 to 405

If more than one assessment is measured within the same visit window, then the record with the latest visit date (from baseline) will be used for that visit window.

4.4.1 Primary Outcomes Endpoint

The primary endpoint for this study is the proportion of patients who develop anti-GH antibodies (i.e., with positive anti-GH antibody screening assay result) after initiating treatment with Nutropin AQ v1.1.

Among all patients with at least one post-baseline screening assay result, the number and percentage of patients who have at least one positive anti-GH antibody screening assay result at any post-baseline visit will be presented. The Clopper-Pearson exact 95% CIs for the percentage will also be provided.

An anti-GH antibody screening assay result is considered positive if the titer is $\geq$ 1.0 mg/L.

4.4.2 Secondary Outcomes Endpoints

The secondary endpoints for this study are as follows:

- Proportion of patients who exhibit growth attenuation is defined as

  (1) initial growth response greater than pretreatment velocity followed by reduction in growth response to below the pretreatment velocity in the subsequent 6- to 12-month treatment period

  or

  (2) reaching $\leq$ 2 cm per year at any scheduled visit: Month 3, 6, 9, or 12

  Please note that for criteria (1), the initial growth response occurs as early as Month 3 and growth attenuation status at post-baseline visit will be evaluated at Months 6, 9, or 12.

  The growth response is based on the annualized growth velocity defined as (height - baseline height)/ (date of height assessment – date of baseline)*365.25 at any post-baseline visit.

  Pretreatment growth velocity is defined as (Baseline height – last historical height) / (date of baseline – date of last historical height)*365.25

  Growth attenuation status at post-baseline visit Tk (=Month 6, Month 9 or Month 12 for k=6, 9, or 12) will be classified as follows:

  Yes: If growth velocity at Tk< pretreatment growth velocity and there is Tj < Tk with growth velocity at Tj > pre-treatment growth velocity, for j<k, and k=6, 9, or 12
  or
  growth velocity $\leq$ 2 cm per year at any scheduled visit: Month 3, 6, 9, or 12

  No: Otherwise.

- 2x2 cross tabulation (with frequencies and percentages) of growth attenuation status (yes or no) and anti-GH antibody status (positive or negative) will be provided for each antibody assay at each visit (Month 6, 9, and12).

  Similar tables of growth attenuation status (yes at any visit from Month 6 to Month 12) and anti-GH antibody status for those who had a positive antibody assay at any follow-up visit (negative at baseline) and those who remained negative throughout the study (including baseline) will also be provided.

- Proportion of patients with neutralizing anti-GH antibody (Nab positive) among those who had positive anti-GH antibody. This analysis will be summarized by visit, as well as cumulatively, for patients who had detectible anti-GH antibody (ATA) at any post baseline visit (with negative antibody assay at baseline), as well as those who remained negative throughout the study (including baseline).

- Annualized 6- to 12- month growth velocities and height SDS. These variables will be summarized separately for patients who were anti-GH antibody-positive at any post-baseline follow-up visit (negative at baseline) and those who were anti-GH antibody-negative at all visits (including baseline).

  The difference between the annualized 6- and 12- month growth velocities will be presented separately for patients who were anti-GH antibody-positive at any post-baseline visit and those who were anti-GH antibody-negative at all visits (including baseline).

  Graphic displays of the individual patient growth patterns (growth velocity); along with the longitudinal antibody profile over the course of the study will be prepared.

  For the above analyses, if there is any patient who appears to have positive anti-GH antibody at baseline, they will be summarized separately from the other groups.

4.4.3 Exploratory Endpoints

This section is not applicable.

4.4.4 Sensitivity Analyses

Sensitivity analyses will be performed on primary and/or secondary outcome measures by excluding patients for the following two types of situations

- Patient 141's birth weight is too light. Based on safety review query, this patient is too small for gestational age. Summary of primary outcome endpoint will be conducted by excluding patient 141 from analyses.

- Patient 107 with negative baseline growth velocity because historical height was recorded by site as slightly greater than baseline height. The site was already permanently closed in 2016 and therefore query couldn't be responded. Summary of secondary outcome endpoints of growth velocity and growth attenuation will be conducted by excluding patient 141 from analyses.

4.4.5 Subgroup Analyses

No subgroup analyses are planned.

4.5 PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

This section is not applicable.

4.6 SAFETY ANALYSES

Safety analyses will be performed on the Safety population.

4.6.1 Exposure of Study Medication

Study medication is open label daily SC injections of Nutropin AQ v1.1 at a dose of up to 0.043 mg/kg/day (0.3 mg/kg/week) for 12 months. Dose adjustments may be made per investigator assessment at the Month 6 visit for changes in weight of at least ± 2 kg from baseline or insulin growth factor-1 (IGF 1) levels, if measured.

The extent of exposure and compliance to study medication for each patient will be calculated as follows:

The extent of exposure to study medication (days) =
(last study treatment date – date of initial dose) +1.

Compliance to study drug between visits =
[1 - number of missed dose / (date of current visit – date of previous visit)] x 100%

The number and percentage of patients who complete study treatment or discontinue prematurely from study treatment will be summarized by reason for discontinuation of study medication. Descriptive statistics (mean, standard deviation, median, minimum, and maximum) of extent of exposure and compliance to study medication will be provided for all patients in the Safety Population.

Study medication administration data and compliance will also be presented in data listings.

4.6.2 Adverse Events

Verbatim descriptions of adverse events (AEs) will be mapped to Medical Dictionary for Regulatory Activities (MedDRA, version 20.1 or newer) preferred terms and System Organ Classes. The severity of AEs will be graded (1 - mild, 2 - moderate, 3 - severe, or 4 - life-threatening) according to Table 1 of the Protocol, and the relationship to study medication (yes vs. no) will be assessed by the investigator according to Table 2 of the Protocol.

All adverse events with an onset date on or after Day 1 of study medication until 28 days after the last dose of study medication will be summarized (with frequency and percentage) by System Organ Class (SOC), preferred term (PT) within SOC, and

severity grade. In the adverse event summaries, a patient having the same event more than once will be counted only once for that event type. For adverse events of varying severity, the highest (worst) grade will be used in the summaries. Missing severity or relationship to study drug will not be imputed.

Summaries of the following adverse events will be provided:

- AEs by SOC and PT
- SAEs by SOC and PT
- AEs by SOC, PT and severity
- AEs by SOC, PT and relationship to study medication
- SAEs by SOC, PT and relationship
- AEs leading to permanently discontinued study medication by relationship to study medication
- Death and cause of death (listing)
- AEs possibly indicative of hypersensitivity by relationship to study medication

All adverse event data will be presented in data listings.

Adverse Event of Special Interest (AESI)

ASEIs will be summarized using the following preferred term and MedDRA codes.

MedDRA Preferred Terms	MedDRA codes from MedDRA v20.1
drug-induced liver injury: Drug-induced liver injury	10072268
suspected transmission of an infectious agent via product	10072754
transmission of an infectious agent via product	10072753

4.6.3 Laboratory Data

Individual serum drug concentration / GH concentration, immunogenicity (anti-GH antibody screening, neutralizing anti-GH antibody, and antibody binding capacity) assay results will be listed, sorted by patient and visit.

The GH concentration at each visit will be summarized for patients in modified ITT population who have serum blood sample assayed for study drug/GH concentration. Descriptive statistics will include the number of observations (n), mean, standard

deviation, coefficient of variation, median, minimum, maximum, geometric mean, and geometric coefficient of variation.

The serum concentration-time profiles in semi-logarithmic (log/linear) scales, growth velocity time-profile and antibody status (anti-GH antibody, neutralizing anti-GH antibodies, antibody binding capacity) for individual patient will be presented.

Patients who became pregnant during the study will be listed with available pregnancy test results.

4.6.4 Vital Signs

Height, weight and their change from baseline values will be summarized in terms of mean, standard deviation, median, minimum, and maximum at each visit. The average of three measurements of height at each visit will be used for analysis and computation of the height SDS and growth velocity. The height and weight data will also be presented in a data listing.

4.6.5 Previous and Concomitant Medications

All verbatim terms for medications recorded in the case report form (CRF) will be coded by preferred term and an anatomic therapeutic class (ATC) term using International Non-proprietary Name (INN) drug terms and procedures. Concomitant medications and prior medications will be summarized separately, based on the safety population, with number (percentage) of patients in each ATC class and preferred drug name. Prior and concomitant medications will also be presented in data listings.

Prior and concomitant medications are defined as follows:

- Prior medication is defined as any medication with start date before the first day of study drug, or checked to be prior to first study drug administration date, or medication with start date missing.

- Concomitant medication/treatment is any medication/treatment with start date on or after the initial dosing of study treatment, whichever occurs first.
  Medication/treatment that started before the first dose and continued during the study treatment will also be counted as concomitant medication/treatment.

4.7 MISSING DATA

Missing data will not be imputed for efficacy outcome measures. All available assessments will be reported with associated number of observations. If a patient discontinues study medication before the end of the 1-year treatment period, their growth rate will be computed for the last 3-month interval visit and used in the calculation of mean growth for that time interval only.

There are two criteria for defining growth attenuation: (1) initial growth response greater than pretreatment velocity followed by reduction in growth response to below the pretreatment velocity in the subsequent 6- to 12-month treatment period, or (2) reaching ≤ 2 cm per year at any scheduled visit: Month 3, 6, 9, or 12.

For criteria (1), if the pretreatment velocity is missing then growth attenuation is missing. Otherwise,

1. If patient discontinued < month 3, the growth attenuation will be missing because the initial growth response cannot be determined for criteria (1).

2. If patient discontinued ≥ month 3 and < month 6, then:

    a. If the height on Month 3 is missing, the growth attenuation will be missing because the initial growth response cannot be determined.

    b. If the height on Month 3 is non-missing, the initial growth response can be determined but the growth attenuation cannot be determined because there is no Month 6 visit to determine the reduction in growth response to below the pretreatment velocity.

3. If patient discontinued ≥ month 6, the growth attenuation will be calculated based on the original definition.

For safety analyses, incomplete onset dates for adverse events and concomitant medications will be imputed according to the following algorithm:

- If the day is missing, the 1st of the month will be used.
- If the day and month are missing, January 1st will be used.

In cases where the imputation of the start/onset date leads to adverse events or concomitant medications occurring before treatment start, the start date will be set to the treatment start date.

Incomplete end dates for non-ongoing adverse events or concomitant medications will be imputed according to the following algorithm:

- If the day is missing, the last day of the month will be used.
- If the day and month are missing, December 31st will be used.

In cases where the imputation of the end date leads to adverse events or concomitant medications ending after date of last contact, the end date will be set to the date of last contact.

4.8 INTERIM ANALYSES

No efficacy interim analyses are planned.

5. REFERENCES

None.

Appendix 1
Protocol Synopsis

PROTOCOL SYNOPSIS

TITLE:	**A PHASE IV, MULTICENTER, OPEN-LABEL STUDY OF THE IMMUNOGENICITY OF NUTROPIN AQ® V1.1 [SOMATROPIN (rDNA ORIGIN) INJECTION] ADMINISTERED DAILY TO NAÏVE GROWTH HORMONE-DEFICIENT CHILDREN *(iSTUDY)***
PROTOCOL NUMBER:	ML29543
VERSION NUMBER:	2
EUDRACT NUMBER:	Not applicable
IND NUMBER:	BB IND 39,305
TEST PRODUCT:	Nutropin AQ® (Somatropin [rDNA origin] injection) (RO6823852)
PHASE:	IV
INDICATION:	Somatropin-naïve prepubertal growth hormone-deficient children
SPONSOR:	Genentech, Inc.

Objectives

The primary objective for this study is to characterize the immunogenicity profile of Nutropin AQ v1.1 when administered as a daily subcutaneous (SC) injection for 12 months (per the U.S. Prescribing Information [USPI] for Nutropin AQ).

The clinical impact of immunogenicity will also be assessed during the course of the study by evaluating patients for functional growth attenuation in association with the development of anti-growth hormone (GH) antibodies.

Study Design

Description of Study

This is a Phase IV, multicenter, open-label, single-arm study of somatropin (rDNA origin) (Nutropin AQ v1.1) in naïve prepubertal children with growth hormone deficiency (GHD). Approximately 80 patients will be enrolled at approximately 30 sites in the U.S. and will be treated with daily SC injections of Nutropin AQ v1.1 at a dose of *up to* 0.043 mg/kg/day (*0.3 mg/kg/week, which is the* approved and indicated dosage *for pediatric GHD*; refer to the USPI for Nutropin AQ) for 12 months. Dose adjustments may be made at the Month 6 visit *for changes in weight and insulin growth factor-1 (IGF-1) levels, if measured.*

Screening will be performed within 28 days prior to Day 1 unless otherwise specified, after which eligible patients will initiate study treatment. *Patients who do not meet eligibility criteria may be re-screened once for this study. If during screening a subject is found to have an abnormal CBC, this test may be repeated within the same screening period at the discretion of the investigator and the subject may be enrolled if the second result is found to be normal per investigator assessment.*

Historical laboratory and radiographic tests will be reviewed prior to obtaining informed consent and initiation of study drug. Study assessments will be scheduled at 3-month intervals following the baseline visit with an additional visit for the Month 1 blood draw, *which can be conducted at home with the help of a home health nurse.* Study assessments will include physical examinations, height and weight measurements, assessment of pubertal status, and collection of serum samples for study-specific laboratory assessments (serum drug concentration and

immunogenicity assessments). Patients will otherwise be treated and followed as determined by their health care provider.

Patients who complete the Month 12 visit will be considered to have completed the study. Patients will be contacted by phone 28 (±3) days after the Month 12 visit to collect adverse events.

Patients who discontinue from the study early will be asked to return to the clinic within 28 *(±3)* days after the last dose of study drug for an early discontinuation visit, which will include the collection of serum samples for serum drug concentration and immunogenicity assessments.

Growth response will be monitored throughout the study. The annualized 6- and 12-month growth velocities for prepubertal patients will be analyzed. Growth velocities of patients who enter puberty during the study will not be included in the 6- and 12-month velocity analyses. However, these patients will remain in the study and their antibody data will be assessed.

Safety assessments will consist of monitoring and recording of all serious and non-serious adverse events as outlined in Section 5.2.

Please see Appendix 1 for the schedule of assessments performed during the study.

Number of Patients

Approximately 80 patients will be enrolled at approximately 30 sites in the study in the U.S.

Target Population

Inclusion Criteria

Patients must meet the following criteria for study entry:

- Signed Informed Consent/Assent Form
  - The parent(s)/guardian(s) must be willing to give written informed consent; and
  - The child may be required to give written informed assent (if able, and dependent on state/Institutional Review Board requirements).
- Willing to adhere to the visit schedules and meet study requirements
  - For patients below the legal age of consent, both child and parent must be able to adhere to dose and visit schedules and meet study requirements.
- Ability to comply with study assessments for the full duration of the study (1 year)
- Male or female age ≥3 years and < 14 years. *The patient may be 14 years old exactly on the day of the first dose of study treatment.*
- Bone age ≤9 years (females) or ≤11 years (males) as determined by X-ray of the left hand and wrist using Greulich and Pyle method and obtained within the *12* months prior to enrollment
- Prepubertal (Tanner I) males and females by physical exam
- Diagnosis of GHD (stimulated GH < 10 ng/mL) by two standard pharmacologic tests obtained up to 12 months prior to informed consent/assent
  - Acceptable GH stimulation tests include: insulin tolerance test (considered two tests if done sequentially with arginine); arginine stimulation test (considered two tests if done sequentially with insulin); glucagon stimulation test; clonidine stimulation test; L-dopa stimulation test; L-dopa + inderal stimulation test (considered one test if done together).
- Normal thyroid function test within the 12 months prior to informed consent/assent
- Normal CBC within the *12* months prior to informed consent/assent
- Documentation of prior height and weight measurements, with height standard deviation score (SDS) ≤ –1.5 (≤5th percentile) for idiopathic isolated GHD patients

Exclusion Criteria

Patients who meet any of the following criteria will be excluded from study entry:

- Any previous rhGH treatment

- Short stature etiologies other than GHD (e.g., untreated hypothyroidism, short stature associated with GH encoding gene mutations, chromosomal defect associated with short stature)
  - Patients with multiple pituitary hormone deficiencies (secondary/tertiary hypothyroidism, central adrenal insufficiency, diabetes insipidus) not associated with an intracranial tumor or central nervous system irradiation must be controlled on replacement medications for ≥6 months prior to study entry. *Hormonal treatments, such as androgens and estrogens to initiate puberty, are not permitted during the study.*
- Acute critical illness or uncontrolled chronic illness, which in the opinion of the investigator and Medical Monitor, would interfere with participation in this study, interpretation of the data, or pose a risk to patient safety
- Chronic illnesses such as inflammatory bowel disease, celiac disease, heart disease, and diabetes
- Bone diseases such as achondroplasia or hypochondroplasia, intracranial tumor, irradiation, and traumatic brain injury
- Patients receiving oral or inhaled chronic corticosteroid therapy (>3 months) for other medical conditions other than central adrenal insufficiency
- Patients who require higher (2× or greater than maintenance) doses of corticosteroids for more than 5 days in the 6 months prior to enrollment in the study
- Patients with active malignancy or any other condition that the investigator believes would pose a significant hazard to the patient if recombinant human growth hormone (rhGH) were initiated
- Females with Turner syndrome (documented with a karyotype or short stature homeobox [SHOX] analysis at any time prior to informed consent/assent), regardless of their GH status
- Prader-Willi syndrome regardless of GH status
- Born small for gestational age regardless of GH status
- Presence of scoliosis requiring monitoring
- Previous participation in another clinical trial or investigation of GH, treatment for growth failure, or treatment with a biologic agent
- Patients with closed epiphyses
- Patients with a known hypersensitivity to somatropin, excipients, or diluent

Length of Study/End of Study

The end of the study is defined as the date when the last patient, last visit (LPLV) occurs. LPLV is expected to occur approximately 12 months after the last patient is enrolled and receives the first Nutropin AQ v1.1 injection.

Outcome Measures

Primary Outcome Measure

The primary outcome measure for this study is the occurrence of anti-GH antibodies in *rhGH*-naïve, prepubertal GHD children treated with Nutropin AQ v1.1.

Secondary Outcome Measures

The secondary outcome measures for this study are to assess:

- The occurrence of functional growth impairment in association with positive antibody formation in patients treated with Nutropin AQ v1.1
- This will be considered in any patient who demonstrates an initial growth response greater than pretreatment velocity after treatment with Nutropin AQ v1.1, but then slows to below pretreatment velocity in the ensuing 6- to 12-month period (or reaches ≤ 2 cm per year)
- The difference between the annualized 6- and 12-month growth velocities for patients who were anti-GH antibody-positive at any post-baseline visit and those who were anti-GH antibody-negative at all visits (including baseline)

- The formation of neutralizing antibodies, and the association between the presence of neutralizing anti-GH antibodies and growth attenuation

Safety Outcome Measures

No specific safety assessments are planned for this study. However, analysis of all safety events will be performed (see Section 6.4).

Investigational Medicinal Product

Test Product

Nutropin AQ v1.1 will be supplied by the Sponsor as labeled study drug using the Nutropin AQ v1.1 NuSpin™ 10 device for the 1-year duration of this study. For information on the formulation, packaging, and handling of Nutropin AQ v1.1, see the pharmacy manual and the USPI for Nutropin AQ.

Nutropin AQ v1.1 will be provided to the patient and administered at a dose of *up to* 0.043 mg/kg/day *(0.3 mg/kg/week). The dose may be* adjusted *at the Month 6 visit per investigator assessment* for*: 1)* a change in body weight of *at least* ±2 kg from baseline *OR 2) a change in IGF-1 level (if tested and the investigator deems a change in dose is clinically indicated)*. The dose of Nutropin AQ v1.1 will be administered via SC injections by the patient or caregiver.

Statistical Methods

Outcomes Analysis

Primary Endpoint

The primary endpoint for this study is the proportion of patients who develop anti-GH antibodies after *initiating* treatment with Nutropin AQ v1.1.

Secondary Endpoints

The secondary endpoints for this study are as follows:

- Proportion of patients who exhibit growth attenuation (defined as initial growth response greater than pretreatment velocity followed by reduction in growth response to below the pretreatment velocity in the subsequent 6- to 12-month treatment period or reaching ≤ 2 cm per year)
  - *Tables of* growth attenuation status (with or without) and anti-GH antibody status (positive or negative) will be provided for *each antibody assay* at each visit.
  - Similar tables *of growth attenuation status and anti-GH antibody status for those* who had *a* positive *antibody assay* at any follow-up visit *and* those *who remained negative throughout the study*
- Proportion of patients with neutralizing antibody among those who had positive anti-GH antibody
  - This analysis will be summarized by visit, as well as *cumulatively*, for patients who had detectible anti-GH antibody at any post-baseline visit, as well as those who *remained negative throughout the study*.
- Annualized 6- and 12-month growth velocities and height SDS will be summarized separately for patients who were anti-GH antibody-positive at any post-baseline follow-up visit and those who were anti-GH antibody-negative at all visits (including baseline). Graphic displays of the individual patient growth patterns along with the longitudinal antibody profile over the course of the study will be prepared. Patients who enter puberty during the study period will be included up to the last visit at which they were determined to be prepubertal.

Additional analyses deemed appropriate for assessing immunogenicity will be described in the Statistical Analysis Plan.

Safety Analysis

Safety analyses will include all patients who received at least one dose of study drug.

Adverse events will be summarized by mapped term, appropriate thesaurus level, and toxicity grade. Summaries (or listings, if appropriate), including investigator assessments of relationship to study drug, will also be produced for each of the following:

- Serious adverse events
- All events leading to the withdrawal of treatment
- All events by severity
- All events possibly indicative of hypersensitivity

Determination of Sample Size

A target enrollment of 80 patients was chosen to allow for a projected 10−15% dropout rate and exclusion of growth data for an estimated 10% of patients who might enter puberty during the study period and leave sufficient patient numbers to compare to previous studies of immunogenicity. This target enrollment was based on enrollment in a previous study (L0368) which planned to enroll 60 patients for the assessment of growth rate and change in height SDS. An initial sample size was chosen to ensure that at least 50 patients would complete the study (L0368 FR 30SEP97; Sec 4.8.4).

This study is expected to yield a minimum of 50 evaluable patients. Given a sample size of 50, and an assumed 50% of patients developing anti-GH antibodies, the 95% confidence interval around this proportion will be (35.53%, 64.47%). This interval was calculated using Clopper-Pearson methodology. Fluctuations in the proportion of patients developing anti-GH antibodies and the increase of evaluable patients will result in more precise confidence intervals. The exact confidence intervals for the scenarios for sample size of 50 and 60 in combination with the proportion of patients who are anti-GH antibody positive of 50% and 20% are listed in the table below.

N	Proportion of Patients Who Are Anti-GH Antibody Positive	95% Confidence Interval (Lower Limit)	95% Confidence Interval (Upper Limit)
50	0.5	0.3553	0.6447
60	0.5	0.3681	0.6319
50	0.2	0.1003	0.3372
60	0.2	0.1078	0.3230

Appendix 2
Schedule of Assessments

Assessment	Screening (Day −28 to Day −1)	Baseline (Day 1) [a]	Month 1 (±3 days)	Month 3 (±3 days)	Month 6 (±3 days)	Month 9 (±3 days)	Month 12 (±3 days) [b]	Safety Follow-Up (±3 days) [c]	Early Discontinuation [d]
Informed consent/assent	x [e]								
Medical history [f]	x								
Eligibility criteria [g]	x								
Demographic data [h]	x								
Limited physical examination [i]	x	x		x	x	x	x		x
Concomitant medications [j]	x	x		x	x	x	x		x
Height and weight	x	x		x	x	x	x		x
Serum sample for serum drug concentration and immunogenicity assessments [k]		x	x	x	x	x	x [l]		x [l]
Optional tests [m]	*x*				*x*		*x*		
NuSpin instruction/ training [n]		x							
Nutropin AQ v1.1 administration [o]		x	x	x	x	x	x		
Nutropin AQ v1.1 dose compliance [p]			x	x	x	x	x		x
Adverse events [q]	*x*	x	x	x	x	x	x	x	x

[a] All baseline assessments should be performed before the first dose of study drug.

[b] The Month 12 visit will serve as the study completion visit. All assessments should be performed after the last dose of study drug for that day.

[c] The patient will be contacted by phone 28 ± 3 days after the Month 12 visit to collect adverse events.

[d] Patients who discontinue from the study early will be asked to return to the clinic within 28 days after the last dose of study drug.

[e] Written informed consent/assent must be obtained and documented before any study-specific screening procedure is performed.

[f] Includes clinically significant diseases, surgeries, prior height and weight measurements, and all prescription medications used by the patient in the 30 days prior to the screening visit.

[g] Historical laboratory tests include two GH stimulation tests with GH response < 10 ng/mL (obtained up to 12 months prior to informed consent/assent; see Protocol Section 4.1.1 for acceptable tests); thyroid function testing (obtained within 12 months prior to informed consent/assent); complete blood counts (obtained within *12* months prior to informed consent/assent); and for females, karyotype or SHOX analysis to exclude the diagnosis of Turner syndrome (obtained any time prior to informed consent/assent). Historical radiographic test includes bone age obtained within *12* months prior to enrollment. If results are not available within 12 months timeframe, the tests that may be done as part of the screening process are CBC and X-ray for bone age. All other tests should be done as part of standard care.

[h] Demographic data will include date of birth, sex, and self-reported race/ethnicity.

[i] Limited physical examinations should be performed as per the standard of care guidelines for a child with GHD undergoing GH therapy. Pubertal status and confirmation of prepubertal status, presence of hip or knee pain, appearance of spine (i.e., appearance of significant scoliosis), and routine fundoscopic examination for absence of signs of intracranial pressure must be recorded. New or worsened clinically significant abnormalities after baseline should be recorded as adverse events, if applicable.

[j] Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements, medications to manage multiple pituitary-hormone deficits as well as other chronic medications) used by a patient from 7 days prior to screening to the study completion/early discontinuation visit.

[k] A single blood sample will be collected at each visit during the treatment period for immunogenicity and drug concentrations. Process each sample for serum and split into four separate tubes for serum drug concentration and immunogenicity assessments (anti-GH antibody screening assay, anti-GH neutralizing antibody assay, and anti-GH antibody binding capacity assay). After baseline, blood samples for immunogenicity and serum drug concentration should be taken at least 12 hours after the last daily dose of Nutropin AQ v1.1 and prior to the next daily dose of Nutropin AQ v1.1.

[l] The last blood sample at Month 12 must be drawn at least 12 hours after the last dose of Nutropin AQ v1.1. In the event of early discontinuation, an early discontinuation visit should be scheduled within 28 days after the last dose of Nutropin AQ v1.1 and a blood sample will be collected for a final assessment of serum drug concentration and immunogenicity.

[m] *Optional tests include a serum sample to test CBC at screening (if results are not available within 12 months prior to screening) and serum samples for IGF-1, which can be drawn at the discretion of the investigator at Month 6 and 12. All optional samples will be analyzed at the local laboratory. Optional tests also include an X-ray to determine bone age eligibility at screening only if the results are not available within 12 months prior to the enrollment date.*

[n] Nutropin AQ v1.1 will be administered via daily SC injections by the patient or caregiver. Instruction and training on Nutropin AQ v1.1 administration with the NuSpin device will be provided by a trained study nurse coordinator.

[o] The first dose (Day 1, baseline visit) should occur after the patient receives dosing administration instruction in the clinic and after the initial blood sample is drawn. Blood samples scheduled after baseline (Months 1, 3, 6, 9, and Month 12 or early discontinuation) should be taken at least 12 hours after the last daily dose of Nutropin v1.1 and prior to the next daily dose.

[p] Assess compliance with daily dosing at each visit after baseline. Reinstruct patients and caregivers, as needed.

[q] After initiation of study drug, all serious adverse events and non-serious adverse events, regardless of relationship to study drug, will be reported until 28 days after the last dose of study drug as provided in this study. After this period, the investigator should report any serious adverse events that are believed to be related to study drug. The Sponsor should be notified if the investigator becomes aware of any serious adverse event that occurs after the end of the adverse event reporting period (defined as 28 days after the last dose of study drug), if the event is believed to be related to prior study drug treatment. The investigator should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the investigator, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all serious adverse events considered to be related to study drug until or trial-related procedures until a final outcome can be reported.

Appendix 3
Programming Codes for Statistical Analysis

Programming of the tables, listings and figures will be performed using SAS Version 9.4, running under UNIX environment. The following table presents the SAS code for the efficacy analysis.

Endpoint	Test	SAS Code

95% Clopper-Pearson confidence interval	95% confidence interval for response rate	ods listing close;
		proc freq data = xxx;
		ods output BinomialCLs=CI BinomialProp=prop;
		table resp / binomial(level = 1 exact)
		out=freq1 (keep=resp count);
		run;
		ods listing;

The following macros will be used for reference to calculate the normal height SDS (z-scores) for this study.

```
*********************************************************************
***  PROGRAM:   htz.sas   Macro
***
***  DESCRIPTION:
***    Macro calculates normal height SDS (z-scores) for NCGS
***     (Growth Hormone marketed product observational study)
***    http://www.cdc.gov/nchs/nhanes/growthcharts/datafiles.htm
***     was http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/datafiles.htm
***
***  ASSUMPTIONS:
***    The macro only uses formats htzml, htzfl, htzmm,
***    htzfm, htzms, and htzfs in /NCGS/lib/formats.sct01.
***    A value is returned only if sex=1 or 2, age>=0,
***    ht>0, and the returned value is -10=<htz<=4.  It
***    uses (and drops) variables beginning with "xzzyww".
***    ("Table" below refers fo values returned from formats.)
***     Temporary variables used:
***    xzzywwa  age(months) in table row just less or = to input age
***    xzzywwa2 age(months) in table row just greater than input age
***    xzzywwi  interpolation factor of age between xzzywwa and xzzywwa2
***    xzzywwl  Box-Cox transformation power         in xzzywwa  row
***    xzzywwl2 Box-Cox transformation power         in xzzywwa2 row
***    xzzywwl  Box-Cox transformation power  interpolated
***    xzzywwm  median                               in xzzywwa  row
***    xzzywwm2 median                               in xzzywwa2 row
***    xzzywwm  median  interpolated
***    xzzywws  generalized coefficient of variation in xzzywwa  row
***    xzzywws2 generalized coefficient of variation in xzzywwa2 row
***    xzzywws  generalized coefficient of variation  interpolated
***
***  INSTRUCTIONS FOR USER:
***    Include the following code with your variables assigned
***    to each of the 4 parameters.
***    %htz(sex=,age=,ht=,htz=) ;
***  INPUT VARIABLES:
***    sex = patient gender variable
```

```
***    age = patient age (yr) variable
***    ht  = patient height (cm) variable
***  OUTPUT VARIABLES:
***    htz = patient height SDS variable
***
***  MACROS:        None
***  INCLUDE CODE:  None
***  FORMATS:       /NCGS/lib/formats.sct01
***                  htzml, htzfl, htzmm, htzfm, htzms, and htzfs
***  AUTHOR:        Ken Dana
***  DATE WRITTEN:  10/2002
***  CHANGES:       (None yet)
*****************************************************************;

 %macro htz(sex=,age=,ht=,htz=) ;
    * reset/initialize temp vars and output parameter, htz ;
    xzzywwa =.; xzzywwl =.; xzzywwm =.; xzzywws =.; xzzywwi=.;
    xzzywwa2=.; xzzywwl2=.; xzzywwm2=.; xzzywws2=.;
    &htz=. ;
    * check input parameters  based on CDC data table limits ;
    if (&sex=1 or &sex=2) and &age>=0 and &ht>0 then do ;
       * calc xzzywwa as age(months) in CDC table just <= macro input age ;
       * (input parameter, age, is in years) ;
       * CDC table age (row) index entries are: ;
       *  half but not whole months, except the limits ;
       *  0, 0.5, 1.5, 2.5, ...., 237.5, 238.5, 239.5, 240 ;
       * if the age is not equivalent to a row age, ;
       *  set xzzywwa to the index just less than age input ;
       if 0<=&age*12<0.5 then xzzywwa=0 ;
        else                  xzzywwa=floor(&age*12+0.5)-0.5 ;
       if &age>=20       then xzzywwa=240 ;
       * get l, m, and s data from CDC table via format lookups ;
       *  for either male (sex=1) or female (sex=2) ;
       if &sex=1 then xzzywwl=input(put(xzzywwa,htzml.),15.) ;
       if &sex=2 then xzzywwl=input(put(xzzywwa,htzfl.),15.) ;
       if &sex=1 then xzzywwm=input(put(xzzywwa,htzmm.),15.) ;
       if &sex=2 then xzzywwm=input(put(xzzywwa,htzfm.),15.) ;
       if &sex=1 then xzzywws=input(put(xzzywwa,htzms.),15.) ;
       if &sex=2 then xzzywws=input(put(xzzywwa,htzfs.),15.) ;
       * if age is not equivalent to an age in the table, interpolate l, m, and s ;
       if &age*12^=xzzywwa & xzzywwa<240 then do ;
          * set xzzywwa2 to age in row with age just greater than macro input age ;
          if 0<=xzzywwa<0.5 then xzzywwa2=0.5 ;
           else                  xzzywwa2=xzzywwa+1 ;
          if xzzywwa2>240   then xzzywwa2=240 ;
          * determine interpolation factor, xzzywwi, between xzzywwa & xzzywwa2 ;
          xzzywwi=(&age*12-xzzywwa) / (xzzywwa2-xzzywwa) ;
          * get l, m, and s from next row in table by xzzywwa2 ;
          *  and interpolate l, m, and s with xzzywwi ;
          if &sex=1 then xzzywwl2=input(put(xzzywwa2,htzml.),15.) ;
          if &sex=2 then xzzywwl2=input(put(xzzywwa2,htzfl.),15.) ;
          xzzywwl= xzzywwl + xzzywwi * (xzzywwl2-xzzywwl) ;
          if &sex=1 then xzzywwm2=input(put(xzzywwa2,htzmm.),15.) ;
          if &sex=2 then xzzywwm2=input(put(xzzywwa2,htzfm.),15.) ;
          xzzywwm= xzzywwm + xzzywwi * (xzzywwm2-xzzywwm) ;
          if &sex=1 then xzzywws2=input(put(xzzywwa2,htzms.),15.) ;
          if &sex=2 then xzzywws2=input(put(xzzywwa2,htzfs.),15.) ;
          xzzywws= xzzywws + xzzywwi * (xzzywws2-xzzywws) ;
```

```
      end ;
   * calculate htz according to CDC formulas ;
   if       xzzywws=0 then &htz=. ; * SAS log() is natural(e) log ;
    else if xzzywwl=0 then &htz= log(&ht/xzzywwm)             /         xzzywws ;
     else                  &htz=  (((&ht/xzzywwm)**xzzywwl)-1)/(xzzywwl*xzzywws) ;
   end ;
* extreme limits on output paramter for multivariate input data problems ;
if .Z<&htz<-10 or &htz>4 then &htz=. ;   * Ken Attie set 2/1991 ;
* ensure temporary variables are not output/kept in the calling data step ;
drop xzzywwa  xzzywwl  xzzywwm  xzzywws  xzzywwi
     xzzywwa2 xzzywwl2 xzzywwm2 xzzywws2 ;
%mend htz ;
```