Prevention of Growth Failure in Turner Syndrome: Long-term Results of Early Growth Hormone Treatment in the "Toddler Turner" Cohort

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Abstract

Introduction: In the landmark “Toddler Turner” study girls who received GH starting at ages 9 months-4 years (Early-Treated [ET] group) had marked catch-up growth and were 1.6±0.6 SD taller than untreated (Early-Untreated [EUT]) girls after 2 years. However, whether the early catch-up growth would result in greater near-adult height (NAH) was unknown. Therefore, this Extension study examined the long-term effects of toddler-age GH treatment on height, pubertal development, and safety parameters.

Methods: Toddler study participants were invited to enroll in a 10-year observational Extension study for annual assessments of growth, pubertal status and safety.

Results: Although the ET group was taller than the EUT group at all timepoints from preschool to maturity, and was significantly taller at onset of puberty($p<0.02$), the difference was not significant at NAH. For the full cohort (ET+EUT combined,n=50) NAH was (mean±SD) 151.2±7.1cm at age 15.0±1.3 years. NAHSDS was >-2.0 for 68% subjects overall and correlated strongly with height SDS at GH start ($r=0.78;p=0.01$), which in turn had a modest inverse correlation with age at GH start (i.e., untreated younger girls were taller for age than older girls ($r=-0.30;p=0.02$). No new safety concerns arose.

Conclusion: Height SDS at NAH was not significantly different between groups due to catch-down growth of ET girls during lapses in GH treatment after the Toddler study, and similar overall GH exposure. Nevertheless, GH treatment in the cohort as a whole prevented ongoing growth failure and facilitated attainment of height within the normal range at key stages of childhood, adolescence, and adulthood.

Introduction

The growth failure that characterizes Turner syndrome (TS) results primarily from haploinsufficiency of the *SHOX* gene on the short arm of the X chromosome [1-6], and is progressive from infancy to adulthood. Average length of full-term babies with TS is approximately 0.7 standard deviations (SD) below the mean for the general population; average untreated height falls below -2.0 SDS by 4 years of age [7], and is below -3.0 SDS in adulthood [8,9]. Average adult heights of large groups of untreated women with TS from various countries are around 143-146 cm, making them about 20 cm below the average heights of their unaffected countrywomen, and about 20 cm below their mid-parental (target) heights [8-13]. Thus, prevention of growth failure and maintenance of height within the population normal range throughout childhood and into adulthood may be considered quintessential goals of care in girls with TS.

Growth hormone (GH) has been approved in the USA since 1996 for treatment of TS-associated short stature, based on studies conducted in the 1980s-1990s in which treatment was typically initiated around 9-10 years of age [14-17]. Because height SDS declines with increasing age in untreated girls, those regimens employed the strategy of delaying estrogen replacement, often until the mid-to-late teen years, to maximize the time available for growth [16,17]. However, subsequent data have highlighted the importance of timely estrogen supplementation not only for feminization, but for other aspects of health and well-being [18-24]. Therefore, in addition to maximizing adult height, the current goals of GH treatment include normalizing stature during the prepubertal years to mitigate early physical and psychosocial barriers, and to allow puberty to begin at a similar age to peers [25,26].

In the randomized, controlled, multicenter “Toddler Turner” trial (parent study of the data reported here), 2 years of GH treatment initiated at an average age of 2 years stimulated significant catch-up growth, returning height to near-average in the preschool years and preventing the ongoing growth failure observed in the untreated controls [35]. However, after completion of the trial important questions remained regarding effects of very early GH treatment on timing and tempo of subsequent growth and pubertal maturation, adult height and long-term safety. To address some of these gaps, children from the Toddler study cohort were invited to participate in a 10-year observational Extension study, providing a unique opportunity to assess the long-term effects of very early GH treatment. Of particular interest was the possibility that girls who received very early GH would attain acceptable heights by their early teen years, allowing pubertal development (either spontaneous or induced) at more physiological ages than had been typically achieved in studies conducted in the 1990s and 2000s.

Subjects, Methods and Treatments

Study design

The data presented in this report derive from a long-term investigation comprised of 3 phases: the 2-year **Toddler Turner** randomized, controlled, trial (RCT); the informal off-study phase between completion of the Toddler study and entry to the Extension, referred to herein as the **Inter-study period**; and the 10-year observational follow-up referred to as the **Extension study (Fig. 1A)**.

Toddler study period

The Toddler study was a two-arm, parallel, open-label, multicenter randomized clinical trial (RCT) of the effect of 2 years of GH treatment (versus no treatment) on linear growth of infants and toddlers with TS (11 US sites; August 1999 to August 2003; NCT00406926)[35]. Inclusion criteria were: karyotype-proven TS; age 9 months to 4 years; normal baseline urinalysis, hemoglobin and TSH; adequate thyroid hormone replacement for those with hypothyroidism. Exclusion criteria were: presence of Y-chromosomal component in the karyotype if gonads were *in situ*, autosomal anomaly, systemic illness or concurrent treatment that might influence growth. Eighty-eight eligible girls were randomized in a 1:1 ratio in an unblinded fashion to either a GH treatment group (50 µg/kg/day Humatrope® [rhGH], Eli Lilly and Company; n=45) or an untreated control group (n=43). Seventy-eight of 88 subjects (89%) completed the 2-year study. The Extension study was not part of the original Toddler study protocol, but was designed after completion of the primary study.

Inter-study period

The Inter-study period between the 2 protocol-defined phases of the study (Toddler and Extension) had no formal protocol. Subjects who had participated as Toddler study controls were provided with complimentary GH for 2 years after completing the original study, in recognition of their 2 years as untreated controls during the RCT; subjects in the GH-treated group of the Toddler study were not provided with GH in the Inter-study period, but were treated at the discretion of their physicians and families during this time. Because of varying departure dates/ages from the Toddler

study and entry dates/ages to the Extension, the duration of each participant's Inter-study period was variable.

Extension study period

The Extension study was a multicenter, 10-year (2005-2015) observational study initiated 2 years after the last subject departed the Toddler study. The protocol was approved by local ethics/institutional review boards and informed consent/assent was obtained from parent(s)/legal guardian(s)/subjects according to local requirements. GH was provided by the sponsor and all Toddler study participants were eligible to enter the Extension study whether or not they had completed the original trial. Investigators were encouraged to follow the pubertal induction regimen described by Davenport [23], with initiation of low-dose transdermal estradiol (E2) at ~11 years of age, followed by slow escalation of E2, addition of progestin after ~3 years, and attainment of adult estrogen dosages after ~4 years. All treatment decisions and dosing for GH and estrogen replacement were at the discretion of the investigators, local endocrinologists, and families.

Data collection in the Extension study

Data collected at Extension study baseline included historical information from the Inter-study period such as illnesses, GH and/or estrogen treatment, concomitant medications, physical examination, auxology, pubertal status, bone age and laboratory data. Data collected at annual visits included medical history since last visit, occurrence of breast development and/or menarche, GH dosing and treatment adherence (missed injections in the week, 3 months, and 12 months prior to the annual visit), concomitant medications, anthropometric measurements, physical examination, Tanner breast and pubic hair stages, bone age x-ray results, laboratory data, and adverse event reports.

Efficacy measures

Puberty was defined as presence of Tanner breast stage 2 or greater (≥B2). Because subjects were seen annually, Tanner stage B2 may have been missed in some patients, in which case B3 was used as the proxy for the start of puberty. Near-adult height (NAH) was defined as the first height measurement obtained after height velocity was ≤2.0 cm/year in the absence of any additional growth-impairing process, or bone age was ≥14.5 years (assessed by two independent readers, blinded to treatment status). Last available height on study was the last height measured prior to subject departure from the study, which may or may not have been equivalent to NAH, as some subjects left before NAH and some continued after.

Safety measures

Targeted questions on case report forms were used to assess occurrence of the following pre-specified adverse events: benign intracranial hypertension, diabetes mellitus or impaired glucose tolerance, ear infections, high blood pressure, hypothyroidism, neoplasia, pancreatitis, scoliosis, and slipped capital femoral epiphysis. Standard reporting of serious adverse events (SAEs) and non-serious, treatment-emergent adverse events (TEAEs) was also performed[1].

Fasting blood samples were drawn at each visit for central laboratory measurements of glucose, insulin, IGF-I and IGFBP-3. HbA1c, oral glucose tolerance,

[1] A serious adverse event (SAE) was defined for this study using US Food and Drug Administration criteria, as an event that was life-threatening or resulted in death, hospitalization or prolongation of hospitalization; congenital anomaly in the offspring of a study subject; persistent or significant disability/incapacity; or required intervention to prevent permanent impairment/damage[36]. A treatment-emergent adverse event (TEAE) was defined as any event that began or worsened after study entry, irrespective of suspected association with treatment.

and fasting lipids were measured at baseline and at approximately age 10, 16 and last visit.

Study populations

For analytical purposes subjects were designated as GH-treated if they received ≥6 months of continuous GH during any phase of the study. Based on their original treatment assignments in the Toddler study, subjects were classified into 2 groups as "Early-Treated" (ET group; those originally randomized to GH) or "Early-Untreated" (EUT group; those originally randomized to the non-GH-treated-control group).

Three populations were defined for analyses: the Safety population included all subjects who had a baseline visit in the Extension study; the Intent-to-Treat (ITT) population included all subjects who had baseline and at least one post-baseline Extension visit; the NAH population was a subset of the ITT population that included all subjects who attained NAH during study. One subject who received no GH at any time (45,X/46,XX karyotype, height at Toddler study entry +1.26 SDS, and height at Extension study entry +1.56 SDS) was excluded from efficacy analyses.

Statistical methods

The primary aim of the Extension study was to evaluate the long-term effect of GH treatment given during very early childhood, by comparing height SDS at NAH of girls who received 2 years' GH in the Toddler study to those who were untreated during that period. To account for potential baseline differences between the ET and EUT groups, a between-group analysis of covariance (ANCOVA) was conducted for NAH, with Toddler study baseline age and baseline height SDS as covariates; results were

considered significant at the 5% level using a 2-sided test ($p<0.05$). Detailed description of power assessment for the primary analysis is provided in Supplemental material.

Mean±SD (or least squares mean/standard error [LSM/SE] for ANCOVA), median, minimum, and maximum (or range) are provided for continuous variables. Categorical variables are summarized as number (percent). No power assessments were made for any analyses other than the primary, so P-values are provided for a limited number of analyses and considered informational only; they should not be used to infer treatment effects or lack thereof. Factors associated with greater NAH were examined using Pearson correlations between NAH SDS and baseline or treatment-related variables (age and height SDS at GH start; duration of GH treatment).

Height SDS values were calculated using US general population standards [37] and statistical analyses were performed using SAS Version 9.1 or higher (SAS Institute Inc., Cary, NC) or SPSS 24 or higher (IBM Corp., Armonk, NY).

Results

Longitudinal data from all three periods of the investigation are included in this report: the original 2-year randomized Toddler study, the informal Inter-study period (variable duration), and the 10-year Extension (**Fig. 1A**).

Demographics

Of 88 eligible Toddler study participants 69 (78%) entered the Extension study and comprise the Safety population (ET,n=36; EUT,n=33); the remaining 19/88 subjects either declined participation or could not be contacted (**Fig.1B**). Sixty-seven of 69 subjects in the Safety population had at least one post-baseline follow-up visit and comprise the ITT population (ET,n=35; EUT,n=32); 51 subjects attained NAH on study

(NAH population: ET,n=25; EUT,n=26). Among 67 girls in the ITT population, 60 (90%) were of Caucasian origin, 46 (69%) had 45,X karyotype, 11 (16%) had 45,X/46,XX karyotype, and 10 (15%) had other karyotypes.

Demographic characteristics of the Extension study ITT population are provided in **Table 1.** The ET and EUT groups were of similar ages at entry to both the Toddler study and the Extension study (~1.9 and ~8.3 years, respectively). The key difference between the groups was the age at initiation of GH: 1.9±0.9 years for the ET group (Toddler study entry), and 4.7±1.8 years for the EUT group, most of whom started GH at the beginning of the Inter-study period (**Table 2A**); because of their older age the EUT group was substantially shorter than the ET group at GH initiation. The 19 subjects who did not participate in the Extension were somewhat shorter at baseline and endpoint of the Toddler study than those who entered (**Supplemental Table**).

GH treatment details and growth responses by treatment group (ET vs. EUT)

GH treatment and overall growth outcomes (GH start to Extension study endpoint) are summarized in **Table 1** (by study period) and **Table 2** (overall)**; Figure 2** illustrates mean height SDS values by treatment group at key timepoints, from Toddler study baseline (average age 1.9 years), to NAH (average age 15.0 years). Individual sections below summarize treatment outcomes during each phase of the study, and overall.

Toddler study period (ITT population)

During the Toddler study all 32 EUT subjects were untreated, whereas all 35 ET subjects received 2 years of GH (50 µg/kg/day), resulting in a marked increase in height

for the ET group and significant between-group height difference of 1.6±0.6 SDS by ANCOVA, p<0.001 [35]. Thus, at the end of the Toddler study at ~3.9 years of age the ET group was on average more than 7 cm taller than the EUT group (**Table 1, Fig. 2**).

Inter-study period (ITT population)

Apart from the Toddler study itself, the Inter-study period (individual subject duration range, 2.6–6.7 years) was the time of greatest difference in growth between the ET and EUT groups. The beginning of the Inter-study period (end of the Toddler study) represented the start of 2 years' complimentary GH for the EUT group, whereas it was the conclusion of protocol-specified GH treatment for the ET group. Thus, 14/35 (40%) ET group subjects were untreated during the Inter-study period for ≥1 yr (range, 1.9–6.4 yr), resulting in substantial post-GH catch-down growth for 11 subjects (average 0.9 SDS decline, range -0.3–-2.2). The remaining 21 subjects in the ET group, who restarted GH within 6 months of departing the Toddler study, as prescribed by their physicians, had an average 0.3 SDS decline over the period. In contrast, the EUT group, which was previously untreated, showed the typical catch-up growth of GH-naïve subjects (average 0.6 SDS height increase; **Figure 2**) as 26/32 subjects (81%) received GH for at least 6 months during this period. As a consequence of the divergent growth patterns during this period (catch-down growth of ET group, catch-up growth of EUT group), there was substantial narrowing of the between-group height difference, from ~1.6 SDS (~7 cm) at the end of the Toddler study, to ~0.6 SDS (~3 cm) at the start of the Extension (**Figure 2,** Toddler Endpoint to Extension Baseline).

Extension study period (ITT population and NAH population)

During the 10-year Extension study, 33/35 girls (94%) in the ET group and 31/32 girls (97%) in the EUT group received GH treatment for an average of 6.1 and 7.4 years respectively (**Table 1**). Sixty of 67 subjects had already received at least 2 years' GH treatment by the time they entered the Extension. Adherence to prescribed GH was generally fair to good: by parent estimate 82-94% of subjects missed 0-2 prescribed injections per week in the 3 months preceding each annual visit; we assumed that the 3-month estimate would be reasonably representative of the full-year's adherence.

Figure 2 demonstrates that the mean height SDS of the ET and EUT groups followed very similar trajectories from Extension study baseline to NAH, with the ET group about 0.4-0.7 SDS taller than the EUT group throughout. Both groups showed a slight increase in height SDS between ~8.3 (Extension baseline) and ~10.0 years of age, followed by a substantial decline (mean ~0.5 SDS) prior to onset of puberty, with little change thereafter. Height SDS changes after onset of puberty were small and the difference carried forward to NAH, where the ET group was somewhat younger and taller than the EUT group (mean: ET, 14.7 years, –1.3 SDS; EUT, 15.3 years, –1.7 SDS; **Table 2B; Fig. 2**).

Study as a whole (Toddler study baseline to Extension study endpoint; *ITT group and NAH group*)

Overall, both treatment groups did well during GH treatment over the prolonged duration of this study, from GH initiation to Extension study endpoint, maintaining average heights within the mid-to-lower part of the population height range. Notably, at the start of GH treatment the EUT group was substantially shorter than the ET group (-

2.2 vs. -1.4 SDS; **Table 2A**), because the EUT group had 2 years of progressive growth failure while untreated during the Toddler study. Consequently, the ET group remained somewhat taller than EUT at each timepoint from the preschool years to maturity.

Although the EUT group started and ended treatment later than the ET group (**Table 2A**) the average total durations and GH dosages for the groups over the whole study were very similar (ITT population, ET:11.0 years, ~0.29 mg/kg/wk; EUT:10.8 years, ~0.30 mg/kg/wk). This similarity of GH exposure largely resulted from the lower rates and greater inconsistency of treatment for the ET group in the Inter-study period (i.e., >6 months interruption: ET,13/35 [37%] vs. EUT,5/31 [16%]). Furthermore, 45% of EUT subjects started GH by age 4.0 years and 84% started by age 6.0 (all ET subjects started GH by age 4).

For the NAH population the combined-group mean NAH of ~151 cm (-1.5 SDS) was attained at 15 years of age (**Table 2B**). The primary efficacy analysis did not demonstrate a significant between-group height SDS difference at NAH when all subjects were included, as specified *a priori* (least squares mean±SEM: ET, –1.35±0.14; EUT, –1.56±0.14; p=0.30 by ANCOVA with age and height SDS at Toddler study baseline as covariates). The results were similar when the EUT subject who received no GH at any time was excluded from the analysis (data not shown). One year after NAH (age 16 years) mean heights were only about 1 cm greater than heights at NAH, indicating that growth had essentially ceased. Mean *changes* in height SDS from GH start to NAH were small (**Table 2B**), and were not appreciably greater when the analysis was limited to subjects whose treatment was uninterrupted from start to finish.

Kaplan-Meier analysis of age at NAH demonstrates a left shift of the curve for the ET group compared with the EUT group, indicating generally somewhat younger attainment of NAH (**Fig. 3A**).

Individual subject outcomes (ITT population and NAH population)

Because of the known variation in outcomes of GH treatment among individual patients, we examined factors associated with greater NAH SDS in our cohort. Correlations between relevant variables were performed for the Extension study cohort as a whole, irrespective of original Toddler study treatment group. There was a strong positive correlation between height SDS at GH start and NAHSDS ($r = 0.78$; $p<0.01$; **Fig. 4A**) and a weaker but still significant inverse (negative) correlation between baseline age and baseline height SDS ($r=–0.30$; $p=0.016$; **Fig. 4B**), indicating that height SDS declined with increasing age prior to start of treatment. There was a negative but non-significant association between age at GH start and NAHSDS ($r = –0.20$; $p=0.17$; **Fig. 4C**). There was no significant association between duration of GH treatment and NAHSDS or change in height SDS from start of GH treatment to NAH.

To evaluate attained heights in context of the normal growth curve for girls, **Figure 5** displays individual subjects' height measurements for the Extension study ITT population at 4 timepoints: baseline and endpoint of the Toddler study, and baseline and endpoint of the Extension. This figure demonstrates the early normalization of height for the ET group, with most height values being above the 5th percentile of the normal curve at the end of the Toddler study, around age 4, compared with very few of the values for the EUT group. However, many EUT subjects caught up later following

initiation of GH during the Inter-study period, such that most of the EUT group's height values were above the 5th percentile of the reference curve by Extension study entry around age 8. For the cohort as a whole more than two-thirds of heights were well within the general population normal range from mid-childhood onward and last available height was above -2.0 SDS for 68% of subjects overall (ET, 19/25[76%]; EUT, 15/25[60%]), and was above the historical mean untreated TS adult height of ~144 cm [17] for 90%.

Pubertal development

An important question raised by this study was whether girls who received GH from the toddler years would attain taller heights in the early teen years than girls treated later, allowing for onset of puberty at more physiological ages than in previous studies. As anticipated, the ET group was significantly taller than the EUT group (ET, -1.0±1.4 SDS; EUT, -1.8±1.2 SDS; p=0.016 by t-test) and about 6 months younger (ET, 12.3±1.8 years; EUT, 12.8±1.8 years) at onset of puberty **(Table 2C)**. Thus the Kaplan-Meier curve for onset of breast development is shifted to the left for the ET group compared with the EUT group, particularly after ~11.5 years of age (**Fig. 3B**).

Of 60 subjects for whom pubertal data were available, breast development occurred spontaneously for 19 (32%); 7 of these subjects received estrogen supplementation 1.3-5.6 years later. Girls whose puberty was induced had breast development an average of >2.5 years later than those who had spontaneous development. Menarche occurred on average about 2 years after onset of breast development overall.

In general, bone age was slightly ahead of chronological age for the ET group until approximately 11 years of age, whereas it was slightly behind chronological age for the EUT group; thereafter the curves were almost superimposed (**Fig. 6A**).

IGF-I and IGFBP-3

During the Toddler study, IGF-I values were >2 SD above the reference population mean[41] at least once for 29% of the ET group, whereas for the EUT group (i.e., non-GH-treated at that time) all values but one were within the normal range, and most were below the population mean. During the Extension, when almost all girls were receiving GH, a small subset of IGF-I and IGFBP-3 values for both groups was >2 SD above the reference population mean **(Figs. 6C-D)**.

Safety

Glucose homeostasis

Fasting blood glucose values were elevated (>5.6 mmol/L;[100 mg/dL]) for 8/67 subjects (12%) at Extension baseline, and for 9%-21% subjects at various post-baseline visits. However, most subjects had normal values at their other visits. Two-hour OGTT glucose values were mildly elevated (8.4-9.2 mmol/L) in 5 subjects (of 148 OGTTs performed during the study overall). Insulin sensitivity was normal (values ≥0.30 on QUICKI; **Fig. 6B**) at baseline and year 1 of the Extension for all but one subject; thereafter, values were subnormal for 3-7 subjects at annual visits up to year 8 and at the endpoint visit.

One subject was hyperglycemic at Extension study baseline, with an abnormal OGTT and HbA1c 6.1%; type 1 DM was diagnosed 15 months later. There were no other reports of DM (either type 1 or type 2) during the Extension. However, metformin

treatment was reported for 3 subjects who had evidence of insulin resistance or mildly impaired glucose tolerance. Lipid results were unremarkable.

Adverse Events

SAEs were reported for 11/69 subjects: 2 events of neoplasia, described below (2 additional neoplasia-related events were not reported as SAEs), 2 surgeries for scoliosis and 3 for congenital anomalies (atrial septal defect, anomalous pulmonary venous connection, and *pterygium colli*), 4 hospitalizations for infectious illnesses (cellulitis, gastroenteritis, and pneumonia [2 events]), and one hospitalization each for headache and gastrointestinal hemorrhage.

Four events of neoplasia (2 in one subject) were reported in 3 subjects in the ET group during the Extension study. A 13-year old girl presented at age 10.5 years with a medulloblastoma, having received ~11 months of GH treatment from age 1.0-2.1 years. The medulloblastoma was treated with surgery, chemotherapy, and cranial irradiation. However, about 2.5 years later she developed acute myeloblastic leukemia, was treated with chemotherapy, and died of complications. The third neoplasia event was a primary mediastinal stage 1 ganglioneuroblastoma in a 6.6-year-old girl. The child had received GH from age ~1.5-2.2 years in the Toddler study, discontinuing GH when she moved out of state. The tumor was diagnosed incidentally on spinal X-rays performed at 6 years of age for scoliosis screening at Extension study entry. After surgical removal of the tumor GH was restarted ~6 weeks later. The fourth neoplasia was a tubulovillous adenoma of the colon in an 11.7-year-old girl who was later found to have a family history of polyposis coli. Apart from a brief suspension of treatment for removal of the adenoma the child received GH consistently from age 1.6 years to age 15.7 years.

Based on “Yes/No” check-box responses on CRFs for pre-specified clinically relevant events, there were no reports of pancreatitis, slipped capital femoral epiphysis, or benign intracranial hypertension. However, the following events were reported for at least one subject during the Extension: otitis media (n=21 subjects), scoliosis (n=21; details below), hypothyroidism (n=11), mild dilatation of the aorta (n=4; details below), type 1 DM (n=1; details below), and hypertension (n=1). In addition to the pre-specified events, TEAEs were reported for 96% of subjects, most commonly typical childhood illnesses (e.g., infections [78%], gastrointestinal disorders [52%], musculoskeletal disorders [48%], respiratory disorders [41%]), and conditions associated with TS (e.g., procedures for orthodontic problems [16%], ear tube insertion [13%], and tympanoplasty [12%]), all considered unlikely to be GH-related. Mild scoliosis was reported for 21/69 (30%) girls; progression resulted in surgical intervention for two. Four subjects were diagnosed with aortic dilatation, using local institutional criteria, at ages 4.9-15.9 years (GH treatment durations, 2.6-10.8 years). One girl with aortic dilatation also had a bicuspid aortic valve; aortic dilatation was reported to resolve in one subject.

Discussion

In 1999, lacking data on early GH treatment in TS, we initiated the Toddler Turner RCT based on the hypothesis that GH treatment in the preschool years could potentially prevent the progressive growth failure that typically begins in infancy in girls with TS [7,35]. As detailed in the initial report from that study, our hypothesis was proven correct in the short term[35]. However, the question remained whether the early gains would be maintained throughout the growth period to adult height.

Long-term follow-up of this unique cohort demonstrated that although the ET girls were on average 7 cm taller than the control group at the end of the RCT, the later-treated (EUT) girls grew well after initiation of GH following the Toddler study (84% began treatment by age 6), narrowing the gap between the groups such that height SDS difference at NAH was not statistically significant. However, the EUT group never fully caught up to the ET group, which remained taller by at least 0.4 SDS throughout childhood and adolescence, to maturity. In addition, the earlier growth of the ET group resulted in significantly taller stature at onset of puberty and the advantage of more physiologically timed pubertal development [57].

Our study provides 3 key observations: first, early GH initiation in girls with TS prevents ongoing growth failure (loss of height SDS) and preserves height potential; second, GH treatment by age 6 should allow attainment of normal adult height the great majority of all patients; third, interruption of GH treatment can undermine its efficacy.

The most important predictor of NAHSDS was height SDS at GH start. In other words, the taller (and generally, younger) the girl is at baseline relative to population standards, the taller she is likely to be after GH treatment. Because approximately half of the height deficit of girls with TS has already occurred by age 3 [43], and height SDS continues to decline with increasing age in untreated girls[7], the earlier treatment is initiated, the briefer will be the duration of growth failure and therefore the smaller the loss of height potential. Translating our finding to individualized patient care indicates that early treatment is likely to be especially relevant in girls who are already short at a young age, because these girls are most likely to attain short adult heights that are physically and/or socially problematic. The strong association between baseline height

SDS and adult height SDS, coupled with the fact that prepubertal growth has the greatest impact on overall height gain [32,42], supports the approach of initiating GH soon after diagnosis of TS to prevent the growth failure that otherwise occurs with advancing age.

In addition to favorable growth outcomes for both the ET and EUT groups, our study provides reassurance regarding long-term GH safety in TS. Most reported adverse events were typical childhood illnesses unrelated to GH (e.g., gastroenteritis) or conditions associated with TS (e.g., surgery for pterygium colli). However, a handful of events warrant discussion. Two neoplastic conditions (medulloblastoma and ganglioneuroblastoma) were of neural origin, and previous data (including case reports as early as the 1960s, before the use of GH) have suggested a predisposition to development of ganglioneuroma, neuroblastoma and related tumors in girls with TS [44-47]. The third *de novo* neoplasm in our study was a colon adenoma in a girl with family history of familial adenomatous polyposis, an autosomal dominant condition that predisposes to colon adenoma and cancer [Beech 2001]. Notably, Danish GH registry data demonstrated a 5-to-7-fold greater relative risk of colon cancer in women with TS vs. general population rates, whereas overall TS cancer rates were not elevated [48,49]. Similarly, a large pediatric post-marketing study found no increase in cancer rates in patients with TS vs. other GH-treated patient groups [50]. The neoplasia events observed in our study therefore appear to fall within the spectrum of malignancies previously reported in TS, irrespective of GH exposure, and did not occur unusually early. Nevertheless, physicians should advise

families that the prescribing information for recombinant GH contains a warning regarding an increased risk of second neoplasm in childhood cancer survivors treated with cranial irradiation who later receive GH treatment.

Aortic dilatation, a serious concern for women with TS, was reported for 4 subjects in our study. However, false positives for children were reported commonly during the 2000s due to failure to index aortic dimensions to body surface area, and absence of TS-specific standards [52]; available data suggest no GH-related increase in risk of this disorder [51].

Although both treatment groups had good outcomes overall, with NAH in the normal range for 76% of the ET group and 60% of the EUT group, our study had a number of limitations. First, the long-term follow-up component of this study was not part of the original design, but was added after the initial Toddler study had completed. While most subjects transitioned smoothly onto GH treatment after the Toddler study, continuing through the Inter-study period to the Extension study, 37% of girls in the ET group had a gap of at least 2 years, both in terms of consistent GH treatment and of prospective, protocol-defined follow-up data collection. Subjects whose GH treatment was interrupted showed considerable catch-down growth while off GH, in some cases reversing the gains made during the Toddler study. This loss of height SDS in the ET group during the Inter-study period was a key factor undermining the between-group difference at NAH. Although the inconsistency of treatment may be viewed as a deficiency, this finding also reflects the real-world experience of some patients in clinical practice, whose treatment may be interrupted for medical, social or financial reasons. Whatever its basis, the detrimental impact of treatment interruption on overall outcome

provides a lesson on the importance of maintaining consistent GH treatment until a satisfactory mature height has been attained, thereby avoiding the phenomenon referred to by Tanner 50 years ago as “regulatory deceleration” [Tanner 1971].

In addition to the impact of lapses in GH treatment for ET subjects after the Toddler study, the second factor eroding the long-term between-group difference was the fact that the cumulative GH exposure of our groups was, in the end, more similar than it was different. Because they started GH about 2-3 years earlier the ET group also finished about 1.5 years earlier, so both treatment groups had similar GH treatment duration to NAH of >10 years (including >7 years before puberty, the time of greatest effectiveness). Thus, with only a few months’ difference in overall GH duration, it is not surprising that height SDS difference was not significant at maturity.

The third issue that may have affected our results was the lack of NAH data for 24% of the Extension cohort (42% of the original study cohort), reflecting the challenges of following very young children for 10-15 years, as also observed in the gold-standard placebo-controlled RCT to adult height [54]. Nevertheless, among 16 subjects (of 66 GH-treated subjects in the ITT population) who did not have NAH available, 10 were at least 14.5 years old at last visit, with average height 151.6 cm -- very similar to that of the NAH analysis population -- supporting the robustness of the efficacy data.

Despite some limitations, our results support our novel approach to clinical management of growth in girls with TS. Our study began with a paradigm

different from traditional GH treatment approaches: rather than aiming to repair height deficits acquired over an extended period of progressive growth failure, we aimed to prevent loss of height relative to peers before it occurs. Consequently, the key measure of efficacy is attained height SDS, rather than change in height SDS. The fact that average height SDS of our ET group during GH treatment was within 1.5 SDS of the general population mean for age from early childhood to maturity provides evidence of the success of our strategy in maintaining normal height for most girls.

Our study is one of only 5 published TS studies that followed a non-treatment parallel group for at least one year [Gravholt,2017], and is unique in demonstrating the dramatic GH responsiveness of preschool-aged girls (35). Comparing our results with those of the 2 published RCTs that maintained untreated/placebo-treated controls to adult height demonstrates that the average height of 152.3 cm for our full NAH cohort was around 4-5 cm greater than mean AH of the GH-treated groups in the earlier studies, in which treatment started at average age ~9-10 years (14,54). Furthermore, 76% of our ET group had NAH SDS within the general population reference range (>-2.0 SDS) at last measurement vs. 40%-50% of treated subjects in the earlier studies, and only 4-5% of non-GH-treated subjects in those studies. In addition, NAH was above the historical untreated adult average for TS of ~144 cm for 90% of our cohort[17].

As we hypothesized over 20 years ago, early initiation of GH can prevent TS-related growth failure. However, treatment should be maintained consistently until a satisfactory final height is attained, otherwise the efficacy may be undermined by catch-down growth after treatment discontinuation. Our approach of preventing ongoing growth failure and preserving the child's genetic height potential by early initiation of GH

treatment is paralleled by the changes in TS treatment guidelines over the past quarter of a century [25,58-60], which have moved progressively away from withholding GH until the patient's height "**has dropped below the fifth percentile of the *normal* female growth curve**" [59]. We concur with the current guidelines' assertion that: "younger age at [GH] treatment initiation, including at least 4 years of treatment prior to puberty. . . [should] allow for age-appropriate induction of feminization, such that the goals for both optimal adult stature and timing of puberty can be achieved" [58]. However, we do not believe it is in the child's best interest to wait until she " . . already has evidence of growth failure (e.g., below 50th percentile height velocity….), [or] ". . is already short….". The height deficits at school entry in untreated girls are large (average ~9–11 cm by age 4–6 years,[7]) and difficult to repair, potentially compounding the significant medical, psychosocial, and educational challenges faced by many girls. Prevention of such deficits is an achievable goal.

In conclusion, our 10-year extension to the Toddler Turner RCT provided good outcomes for both treatment groups but, largely because of lapses in treatment for the ET group after the initial 2 years, we did not demonstrate a significant impact on NAH of starting GH before 4 years of age vs. starting 2-3 years later. Nevertheless, girls who were taller for age at start of treatment attained greater NAH than girls who were shorter for age, indicating that in general younger GH initiation before substantial loss of height potential has occurred results in the best height outcome at NAH. Based on these findings, we conclude that GH initiation before the age of ~6 years is effective in preventing

growth failure, maintaining growth potential and facilitating attainment of adult height within the normal range in girls with TS. Early treatment also offers the potential advantages of normal height at key developmental timepoints such as school entry and onset of puberty, providing the opportunity for girls with TS to experience a pattern of growth similar to that of their non-TS peers.

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Statement of Ethics

The study protocol (Clinical Trial Registry No. NCT00191113) was approved by the ethics review boards of the participating institutions and conducted according to the standards of the World Medical Association Declaration of Helsinki. Written informed consent for all study-specific procedures was obtained from the subjects' parents or legal guardians and assent was obtained from participants according to institutional guidelines.

Disclosures

CAQ, EE, RLH, FU, KR, ST, and MLD declare no conflicts of interest. PYF has a research contract from Pfizer. MG has a research contract from Novo Nordisk; is an advisory board member for Adrenas, Daiichi Sankyo, Ferring, Neurocrine Biosciences, Novo Nordisk, Nutritional Growth Solutions, Pfizer, QED, and Spruce Biosciences; is a member of data safety monitoring boards for Ascendis, Millendo, and Tolmar and

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Contributions

CAQ, MLD, BJC, and AJZ conceived of and designed the study. BJC, AJZ, CAQ and MLD designed the statistical analyses. CAQ and MLD drafted the manuscript and interpreted the data. All authors acquired the data and provided critical review and approval of the manuscript.

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Figure Legends

Fig. 1A. Study flow diagram. Eighty-eight girls with karyotype-proven TS were randomized at baseline of the 2-year Toddler study in a 1:1 ratio to GH treatment (GH Group, n=45) or non-GH-treated control (Control Group, n=43). After completing the Toddler study, Control subjects were offered 2 years' complimentary GH in recognition of their 2 years on non-treatment during the controlled phase. The Inter-study period between the end of the Toddler study and the Extension study was of variable duration for individual subjects (2.6-6.7 years), depending on timing of departure from the Toddler study and entry to the Extension study. Of 88 Toddler study subjects, 69 entered the Extension study and were classified according to their original Toddler study treatment as Early Treated (ET group, n=36; original GH group) or Early Untreated (EUT, n=33; original Control group). Fifty-one subjects attained near-adult height (NAH) on study (ET, n=25; EUT, n=26). One EUT subject who received no GH at any time was excluded from efficacy analyses.

Fig. 1B. Subject disposition in the Extension study. Of 88 Toddler study subjects, 69 entered the Extension study (Safety population), 67 had at least 1 post-baseline visit (Intent-to-Treat population), and 51 attained NAH on study (defined as the first height measurement after height velocity was ≤ 2.0 cm/year ***or*** bone age was ≥ 14.5 years). One EUT subject who received no GH at any time was excluded from efficacy analyses.

Fig. 2. Mean height SDS from Toddler study baseline to Extension study endpoint (ITT population). The ET group is shown as filled circles with solid line and the EUT group as crosses with dashed line. For both groups each point represents the mean age (X axis) and mean height SDS (Y axis) for subjects in the ITT population at each key timepoint. Horizontal lines beneath the treatment group lines represent the range of GH start ages for the two groups (ET: 0.8–4.0 years, solid line; EUT: 1.7–9.9 years, dashed line). The 5 horizontal dotted lines at the base of the graph represent the ranges of ages at each of the key timepoints (Toddler baseline [TB], 0.8–4.0 years); (Toddler endpoint [TE], 1.7–6.0 years); (Extension baseline [EB], 6.1–11.1 years); (Puberty onset [≥B2], 7.8–16.0 years); (GH endpoint [GH end], 11.4–18.4 years, excluding 3 girls who received no GH during the Extension).

Fig. 3. Kaplan-Meier curves. A. Chronological age at attainment of near-adult height (NAH population). **B.** Chronological age at onset of puberty (Tanner breast stage ≥2; ITT population).

Fig. 4. Pearson correlations. A. Height SDS at GH start vs. near-adult height SDS ($r=0.78$, $p<0.01$). **B.** Age at GH start vs. height SDS at GH start ($r=–0.30$, $p=0.02$). **C.** Age at GH start vs. near-adult height SDS ($r=–0.20$, $p=0.17$).

Fig. 5. Heights at Toddler study baseline and endpoint and Extension study baseline and endpoint (ITT population). Solid lines depict the 5^{th}, 50^{th}, and 95^{th} percentiles of the United States Centers for Disease Control growth chart for girls

(https://www.cdc.gov/growthcharts/percentile_data_files.htm; Accessed on June 23, 2017); shaded area represents the mean ± 1 SD for Turner syndrome according to the Ranke standard (Ranke MB *et al.* "Spontaneous growth in Turner's syndrome" *Acta Paediatr Scand.* 1988;Suppl 343:22-30). Heights at Toddler study baseline and endpoint (open and filled circles, respectively) and Extension study baseline and endpoint (open and filled squares, respectively) for the Early Treated group (left graph, blue symbols) and for the Early Untreated group (right graph, red symbols). Note that some early departures mean that endpoint was not at NAH for all subjects. Red 'x' symbols represent the subject in the EUT group who was tall at baseline and received no GH at any time.

Fig. 6. A. Bone age vs. chronological age (mean) by treatment group.
B. Quantitative insulin sensitivity check index (QUICKI) values (mean ± SD) by age and treatment group, with mean years of GH treatment at each integer age. Dotted lines represent the mean and SD of QUICKI in healthy prepubertal children [40].
C. IGF-I values by treatment group (Safety population), plotted against the mean ± 2 SD for a cohort of healthy girls [41]. Over the duration of the combined study periods there were 380 values for the ET population (n=36) and 369 values for the EUT population (n=33). **D.** IGFBP-3 values by treatment group (Safety population), plotted against the mean ± 2 SD of the range for Danish girls [61]. Over the duration of the combined study periods there were 380 values for the ET population (n=36) and 370 values for the EUT population (n=33).

Table 1. Demographic data for the ITT population

	Early Treated	Early Untreated	All
Toddler Study Baseline			
	n = 35	**n = 32**	**n = 67**
Age, yr	1.90 ± 0.94	1.92 ± 1.02	1.91 ± 0.97
Bone age, yr	1.93 ± 0.85	1.79 ± 0.95	1.87 ± 0.89
Height, cm	78.1 ± 8.1	77.3 ± 8.9	77.7 ± 8.4
Height SDS	-1.39 ± 1.10	-1.62 ± 1.08	-1.50 ± 1.09
Mid-parental height, cm	165.0 ± 5.2	163.9 ± 4.9	164.5 ± 5.0
Toddler Study Endpoint			
	n = 35	**n = 32**	**n = 67**
Age, yr	3.88 ± 0.99	3.91 ± 1.09	3.89 ± 1.03
Bone age, yr*	4.19 ± 1.36	3.34 ± 1.09	3.78 ± 1.30
Height, cm**	98.7 ± 7.5	91.5 ± 7.6	95.3 ± 8.3
Height SDS**	-0.30 ± 1.14	-2.08 ± 1.23	-1.15 ± 1.48
GH duration, yr	1.99 ± 0.23	NA	NA
Inter-study Period			
	n = 35	**n = 32**	**n = 67**
Subjects treated, n (%)[a]	26 (74%)	26 (81%)	52 (78%)
GH duration, yr	2.83 ± 1.89	3.42 ± 1.78	3.11 ± 1.85
Extension Study Baseline			
	n = 35	**n = 32**	**n = 67**
Age, yr	8.23 ± 1.18	8.35 ± 1.27	8.29 ± 1.22
Bone age, yr	8.76 ± 1.57	8.28 ± 1.56	8.53 ± 1.57
Height, cm	124.0 ± 10.1	121.0 ± 9.9	122.6 ± 10.0
Height SDS*	-0.84 ± 1.24	-1.45 ± 1.23	-1.13 ± 1.27
Extension Study Endpoint			
	n = 35[b]	**n = 31**[c]	**n = 66**[b,c]
Age, yr*	15.19 ± 1.63	16.10 ± 1.22	15.62 ± 1.51
Height, cm	151.7 ± 8.2	150.9 ± 6.2*	151.3 ± 7.3
Height SDS	-1.36 ± 1.10	-1.78 ± 0.99*	-1.56 ± 1.07
GH duration, yr	6.13 ± 2.37	7.38 ± 1.25	6.72 ± 2.01

Data are mean ± SD. *p<0.05; **p<0.001 for comparison of means between ET and EUT groups by ANOVA or 2-tailed t test. NA = not applicable because EUT group was untreated during Toddler study. [a]Includes all subjects who received at least 6 months' GH treatment during the Inter-study period; treatment was at the discretion of families and physicians, so some subjects elected to remain untreated; [b]Includes two ET subjects who received no further GH treatment after completing Toddler study; [c]Excludes one EUT subject who received no GH at any time.
Abbreviations: BMI=body mass index; cm=centimetres; SDS=standard deviation score; yr=year.

Table 2A. GH treatment and growth parameters for study as a whole (GH start to Extension study end): *ITT Population*

	Early Treated	Early Untreated	All
	n = 35[a]	**n = 31[b]**	**n = 66[a,b]**
Age at GH initiation, yr*	1.90 ± 0.94	4.66 ± 1.78[c]	3.20 ± 1.97
Height at GH initiation, cm	78.1 ± 8.1	95.5 ± 12.1	86.3 ± 13.4
Height SDS at GH initiation	-1.39 ± 1.10	-2.17 ± 1.03	-1.75 ± 1.13
Age at GH endpoint, yr	14.20 ± 3.10	15.74 ± 1.14	14.92 ± 2.50
Height at GH endpoint, cm[d]	147.0 ± 15.9	149.9 ± 6.5	148.3 ± 12.4
Height SDS at GH endpoint[d]	-1.15 ± 1.26	-1.79 ± 1.01	-1.45 ± 1.18
GH treatment duration[d]	10.96 ± 3.39	10.80 ± 2.03	10.88 ± 2.81
Change in height SDS	0.03 ± 0.87	0.39 ± 0.69	0.20 ± 0.81

Data are mean ± SD. *p<0.05 for comparison of means between ET and EUT groups by ANOVA. [a]Includes two ET subjects who received no further GH treatment after completing Toddler study; [b]Excludes one EUT subject who received no GH at any time; [c]GH initiation for EUT population was at start of Inter-study period for 26/31 subjects and at Extension start for remaining 5/31; [d]Endpoint is not equivalent to NAH because some subjects in ITT population left before, or continued after, NAH.

Abbreviations: BMI=body mass index; cm= centimetres; SDS=standard deviation score; yr=year.

Table 2B. GH treatment and growth parameters for study as a whole (GH start to Extension study end): *NAH Population*

	At NAH		
	Early Treated	**Early Untreated**	**All**
	n = 25[a]	**n = 25[b]**	**n = 50[a,b]**
Age at GH initiation, yr*	1.99 ± 0.98	4.62 ± 1.79[c]	3.30 ± 1.95
Height at GH initiation, cm	78.7 ± 8.0	95.7± 12.2	87.2 ± 13.3
Height SDS at GH initiation	-1.41 ± 1.21	-2.06 ± 1.06	-1.73 ± 1.17
Age at NAH, yr[c]	14.65 ± 1.23	15.32 ± 1.20	14.99 ± 1.25
Height at NAH, cm	151.9 ± 7.6	150.5 ± 6.6	151.2 ± 7.1
Height SDS at NAH	-1.31 ± 1.13	-1.73 ± 1.05	-1.52 ± 1.10
GH treatment duration to NAH	11.01 ± 1.13	10.40 ± 2.19	10.71 ± 2.47
Change in height SDS to NAH	0.09 ± 0.84	0.33 ± 0.67	0.21 ± 0.76
	At Last Visit at or Beyond NAH		
Age at last visit, yr[c]	15.80 ± 1.12	16.24 ± 1.28	16.02 ± 1.21
Height at last visit, cm	153.3 ± 6.7	151.2 ± 6.6	152.3 ± 6.7
Height SDS at last visit	-1.37 ± 1.09	-1.72 ± 1.05	-1.55 ± 1.08
GH treatment duration to last visit, yr	11.75 ± 2.91	10.95 ± 2.11	11.35 ± 2.55
Change in height SDS to last visit	0.03 ± 0.77	0.33 ± 0.72	0.18 ± 0.75

Note: NAH was defined for this study as the first height measurement obtained after height velocity was ≤2.0 cm/ or bone age was ≥14.5 years. Data are mean ± SD. *p<0.05 for comparison of means between ET and EUT groups by ANOVA. [a]Includes two ET subjects who received no further GH treatment after completing Toddler study; [b]Excludes one EUT subject who received no GH at any time; [c]GH initiation for EUT population was at start of Inter-study period for 26/31 subjects and at Extension start for remaining 5/31.
Abbreviations: BMI=body mass index; cm=centimetres; SDS=standard deviation score; yr=year.

Table 2C. Pubertal parameters: *ITT population*

	Early Treated N=35	Early Untreated N=31	Overall N=66
Age at ≥B2, yr[a]	12.28 ± 1.78, n=30 (7.78–16.00)	12.78 ± 1.82, n=30 (8.91–15.73)	12.53 ± 1.80, n=60 (7.78–16.00)
Spontaneous[b]	10.59 ± 1.63, n=10	10.56 ± 1.15, n=9	10.57 ± 1.39, n=19
Induced[c]	13.12 ± 1.15, n=20	13.74 ± 1.03, n=21	13.44 ± 1.12, n=41
Height SDS at ≥B2*	-1.02 ± 1.35, n=30 (-3.69–1.48)	-1.84 ± 1.19, n=30 (-4.44–0.66)	-1.43 ± 1.33, n=60 (-4.44–1.48)
Age at estrogen start, yr	12.16 ± 1.08, n=24 (10.39–14.80)	12.50 ± 1.29, n=26 (10.90–16.00)	12.34 ± 1.19, n=50 (10.39–16.00)
Age at menarche, yr	13.94 ± 1.43, n=20 (10.67–16.17)	14.36 ± 1.81, n=18 (10.88–18.54)	14.14 ± 1.62, n=38 (10.67–18.54)
Time from ≥B2 to menarche, yr	1.95 ± 1.11, n=19 (0.13–4.00)	2.15 ± 1.53, n=18 (-0.35–5.75)	2.04 ± 1.31, n=37 (-0.35–5.75)

Data are mean ± SD (range); n=number with data available for this parameter. *p=0.016 for between-group difference by 2-tailed t test. Data were unavailable for 4 subjects who discontinued the study before onset of puberty (ages: 11.4-12.6 at last observation) and 2 subjects for whom B2 could not be ascertained. [a]Defined as age at first recorded date of breast development ≥ Tanner stage 2 (B2). [b]Defined as breast development ≥ Tanner stage 2 reported prior to estrogen exposure; [c]defined as breast development reported after estrogen exposure.

Long-term Results of Very Early Growth Hormone Treatment in the “Toddler Turner” Cohort

FIGURES

October 2020

Figure 1a

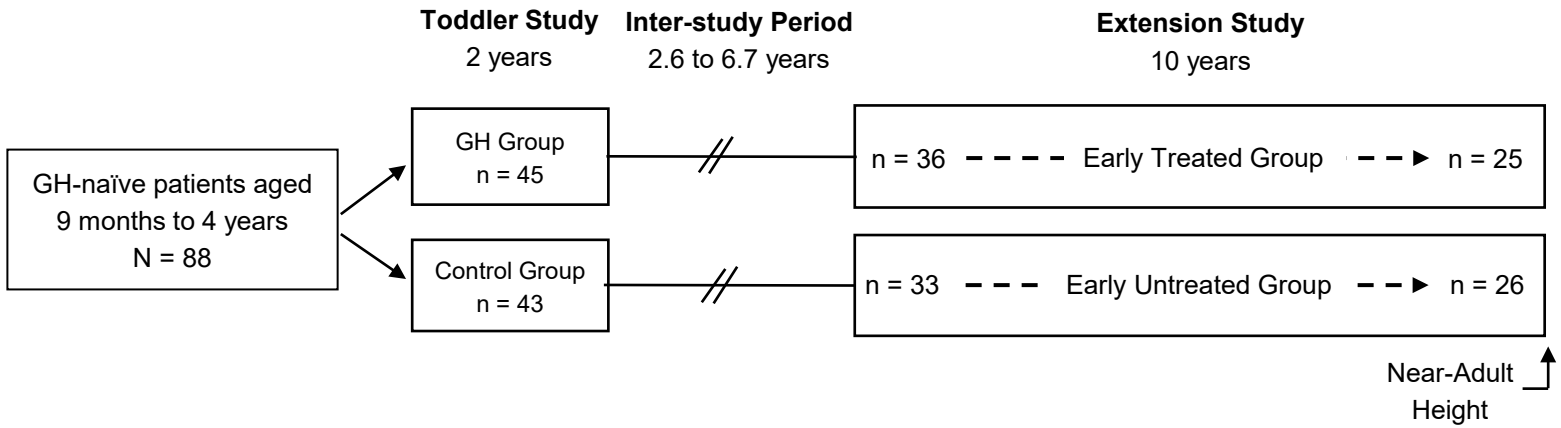

Toddler Study
2 years
Inter-study Period
2.6 to 6.7 years
Extension Study
10 years
GH-naïve patients aged
9 months to 4 years
N = 88
GH Group
n = 45
Control Group
n = 43
n = 36
Early Treated Group
n = 25
n = 33
Early Untreated Group
n = 26
Near-Adult
Height

Figure 1b

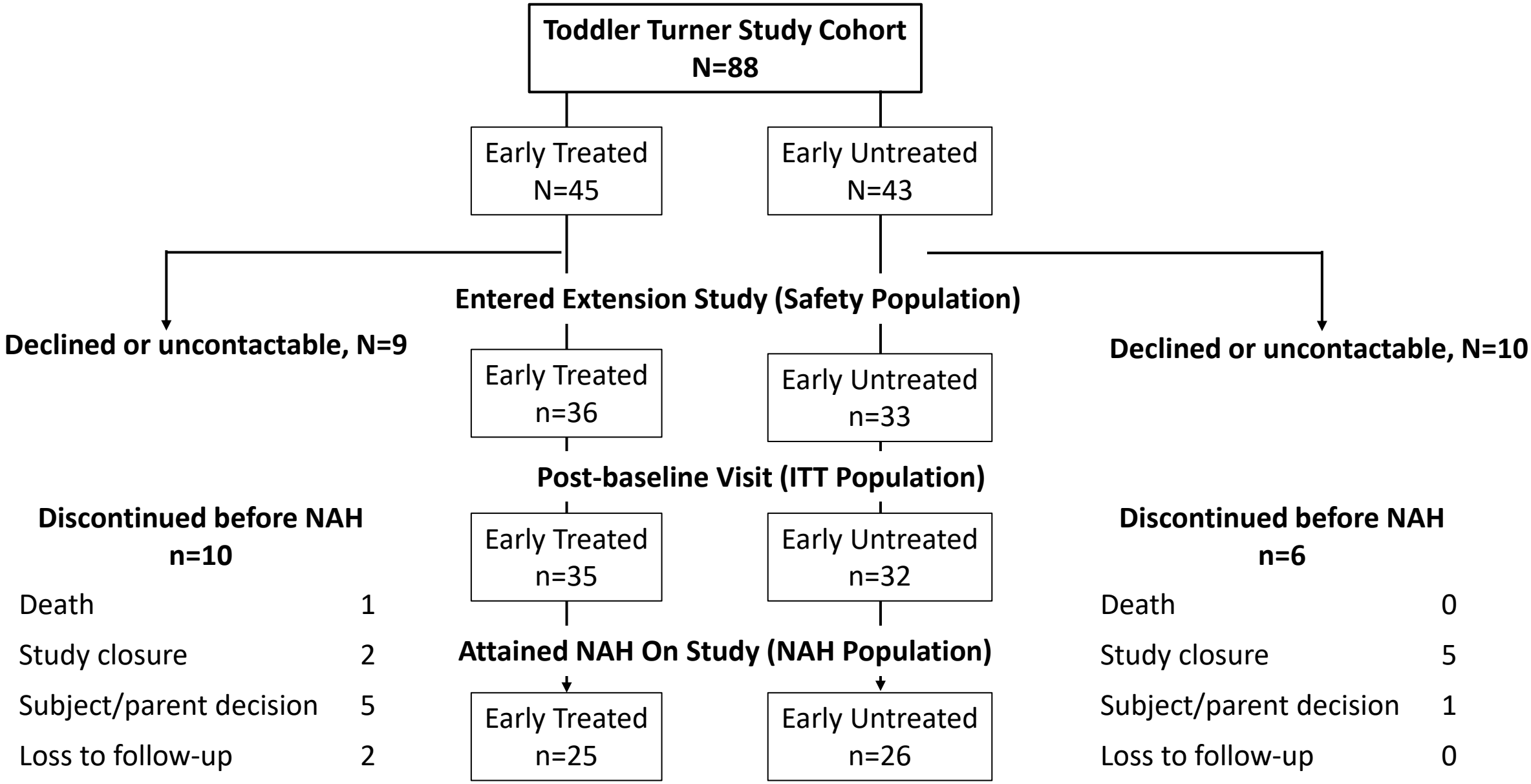

Toddler Turner Study Cohort
N=88
Early Treated
N=45
Early Untreated
N=43
Entered Extension Study (Safety Population)
Declined or uncontactable, N=9
Declined or uncontactable, N=10
Early Treated
n=36
Early Untreated
n=33
Post-baseline Visit (ITT Population)
Discontinued before NAH
n=10
Death 1
Study closure 2
Subject/parent decision 5
Loss to follow-up 2
Early Treated
n=35
Early Untreated
n=32
Discontinued before NAH
n=6
Death 0
Study closure 5
Subject/parent decision 1
Loss to follow-up 0
Attained NAH On Study (NAH Population)
Early Treated
n=25
Early Untreated
n=26

Figure 2

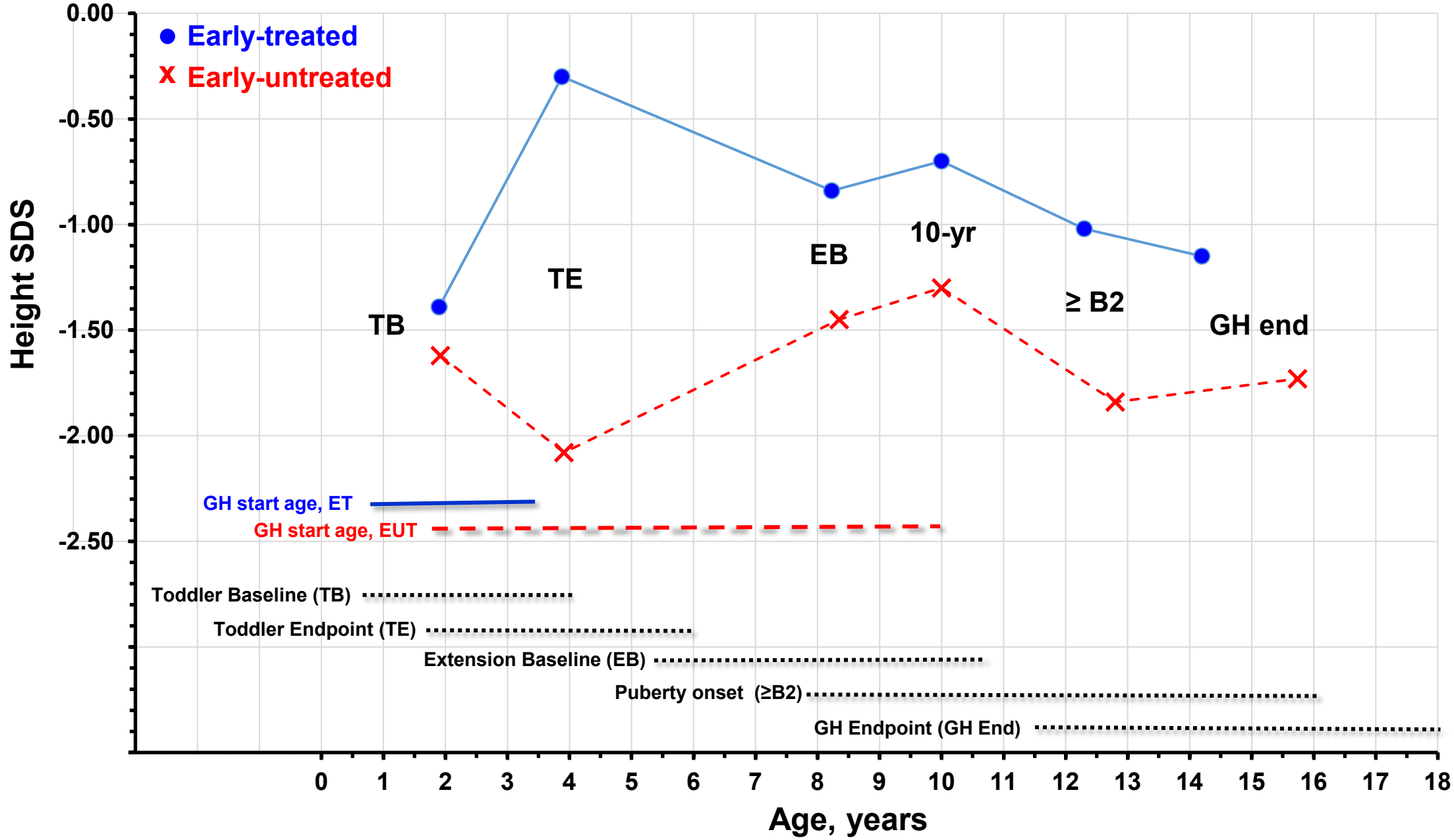

Early-treated
Early-untreated
Height SDS
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
TB
TE
EB
10-yr
≥ B2
GH end
GH start age, ET
GH start age, EUT
Toddler Baseline (TB)
Toddler Endpoint (TE)
Extension Baseline (EB)
Puberty onset (≥B2)
GH Endpoint (GH End)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Age, years

Figure 3a

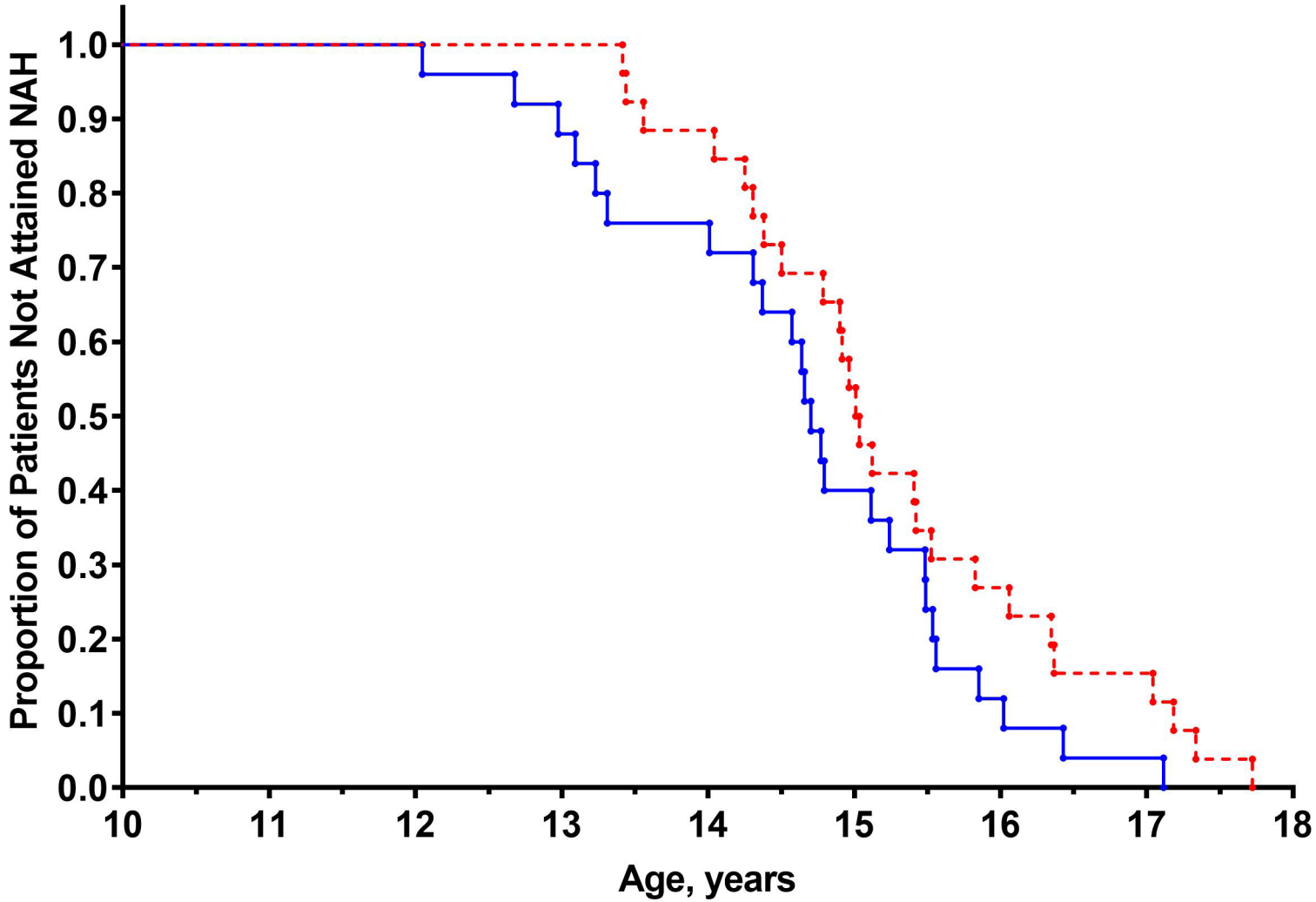

Proportion of Patients Not Attained NAH
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10
11
12
13
14
15
16
17
18
Age, years

Figure 3b

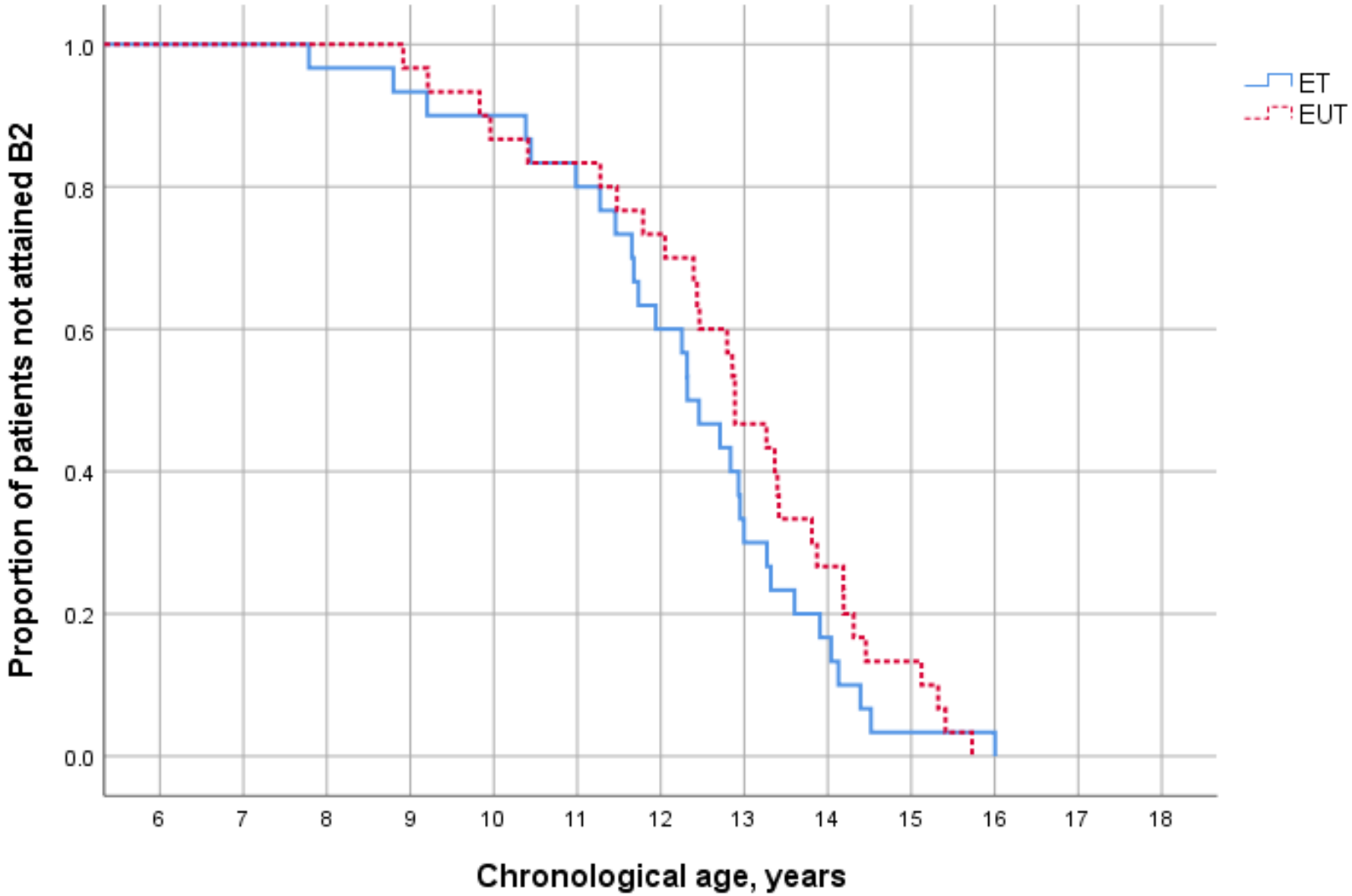

Proportion of patients not attained B2
1.0
0.8
0.6
0.4
0.2
0.0
6
7
8
9
10
11
12
13
14
15
16
17
18
Chronological age, years
ET
EUT

Figure 4, see separate file

Figure 5

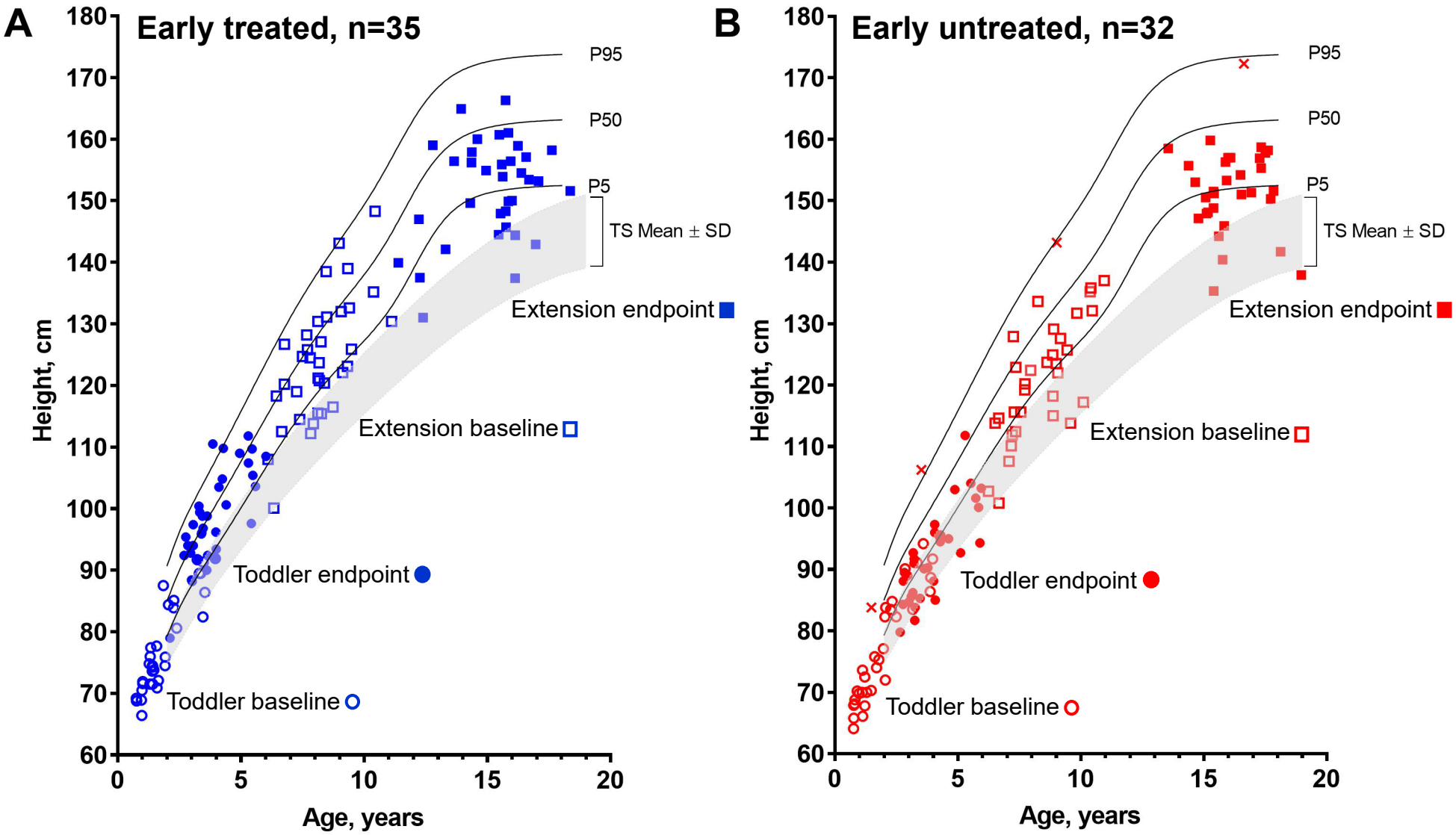
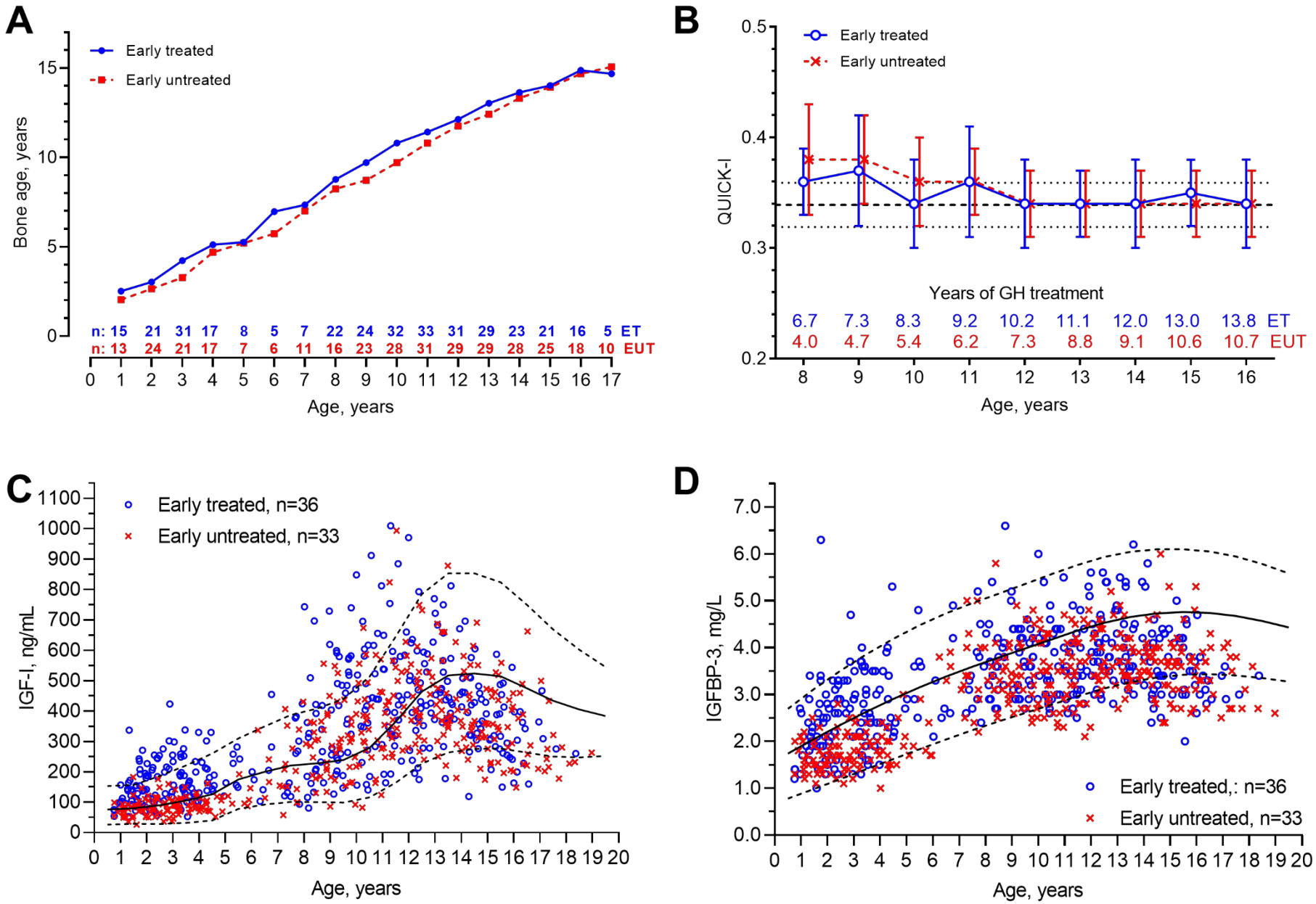

A
Early treated, n=35
B
Early untreated, n=32
P95
P50
P5
TS Mean ± SD
Extension endpoint
Extension baseline
Toddler endpoint
Toddler baseline
Height, cm
Age, years
180
170
160
150
140
130
120
110
100
90
80
70
60
0
5
10
15
20

Figure 6


A
Early treated
Early untreated
Bone age, years
n: 15 21 31 17 8 5 7 22 24 32 33 31 29 23 21 16 5 ET
n: 13 24 21 17 7 6 11 16 23 28 31 29 29 28 25 18 10 EUT
Age, years
B
Early treated
Early untreated
QUICK-I
Years of GH treatment
6.7 7.3 8.3 9.2 10.2 11.1 12.0 13.0 13.8 ET
4.0 4.7 5.4 6.2 7.3 8.8 9.1 10.6 10.7 EUT
Age, years
C
Early treated, n=36
Early untreated, n=33
IGF-I, ng/mL
Age, years
D
IGFBP-3, mg/L
Early treated,: n=36
Early untreated, n=33
Age, years