



Immunogenicity Profile of Lonaepsomatropin During Long-Term Treatment in Children With Growth Hormone Deficiency

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Abstract

Monitoring and evaluation of immunogenicity is important for biological therapeutics due to the potential impact of antibodies on safety and efficacy. A comprehensive immunogenicity assessment was conducted to support clinical development of lonaepsomatropin, a prodrug of somatropin, administered once weekly. Lonaepsomatropin is approved in the United States for pediatric and adult growth hormone deficiency (GHD), and in other countries for pediatric GHD. Lonaepsomatropin consists of somatropin, an inert methoxy polyethylene glycol carrier (mPEG), and a proprietary TransCon[®] linker that transiently binds somatropin to the carrier. Four dedicated immunoassays were developed, validated, and applied in a risk-based tiered approach to rigorously assess the immunogenicity of lonaepsomatropin in children with GHD. Samples collected from 298 children with GHD treated with lonaepsomatropin for up to six years were analyzed for anti-drug antibodies (ADAs) against lonaepsomatropin (intact prodrug), released somatropin, and mPEG. Treatment-emergent anti-lonaepsomatropin, anti-somatropin, and anti-mPEG binding antibodies were detected in 4.7%, 5.4%, and 0.7% of treated children, respectively. ADA responses were low titer, transiently detected, and primarily observed within the first year of treatment, and no neutralizing antibodies were detected. These findings support the long-term low immunogenicity of lonaepsomatropin in children with GHD, consistent with somatropin. Importantly, the multi-assay tiered strategy applied here was robust and reproducible, enabling sensitive detection of ADAs against the intact prodrug, mPEG, and released somatropin across trials, and demonstrated its clinical applicability for evaluating a complex long-acting growth hormone like lonaepsomatropin.

Keywords anti-drug antibodies · growth hormone · immunogenicity · lonaepsomatropin · TransCon hGH

Introduction

Growth hormone (GH) is essential to overall endocrine health, promoting growth and maintaining normal body composition, cardiovascular function, and metabolism (1–3). Pediatric growth hormone deficiency (GHD) is a rare endocrine disorder characterized by growth failure and maturation delays (4). The primary treatment for pediatric GHD is replacement of the deficient GH (1). The initial derivation

of human GH (hGH) was from cadaveric human pituitary glands, but concerns about the potential transmission of diseases, along with limited availability, led to the development of recombinant hGH (rhGH) in 1985 (1).

For over 30 years, the standard of care for pediatric GHD has been daily subcutaneous (SC) injections of somatropin (2). While effective, daily administration is burdensome to families, particularly for children with needle phobia, and can lead to treatment fatigue and reduced medication adherence. These factors underscore the need for alternative administration methods to enhance outcomes and satisfaction (5–7). To mitigate these adherence challenges, long-acting growth hormone (LAGH) therapies have been developed as an alternative to daily administration. These products were designed to reduce dosing frequency while preserving comparable efficacy and safety profiles to daily somatropin. To develop a LAGH, two basic approaches have been employed: (a) combining unmodified GH with

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a prolongation technology, or (b) modifying GH to extend its half-life (8). Daily somatotropin is associated with a low incidence of immunogenicity, and approaches that preserve the native GH structure are anticipated to maintain a similarly low immunogenic profile. These distinctions highlight that LAGH products are not interchangeable, and different strategies may have varying pharmacokinetic properties and immunogenicity profiles. By leveraging their pharmacokinetic properties, LAGHs can improve adherence and overall treatment outcomes (2, 7).

Lonaepsomatropin (TransCon® hGH) is a prodrug of somatotropin administered SC once weekly. It was approved in 2021 by the Food and Drug Administration for pediatric GHD and in 2025 for adult patients with GHD. Additionally, it is also approved in the EU and other countries for pediatric GHD (9–11). Lonaepsomatropin uses TransCon® technology, which transiently links somatotropin to an inert carrier (Fig. 1) (2, 9). The carrier has a shielding effect that minimizes renal excretion and receptor-mediated clearance of somatotropin. As a result, upon injection, active, unmodified somatotropin, with the identical 191 amino acid sequence and size (22 kDa) as endogenous growth hormone (and daily somatotropin products) is released in a sustained manner in response to physiological conditions in the body, thereby supporting once-weekly dosing (2, 12).

Characterizing immunogenicity and its potential clinical effects is important for the clinical development of biological products. Low incidence of anti-drug antibodies (ADAs)

and absence of neutralizing ADAs are key characteristics of safe and effective biologics (7, 13). This paper describes the immunogenicity strategy and assays applied, as well as the results generated during late-stage clinical development of lonaepsomatropin in pediatric GHD.

Materials & Methods

Clinical Trial Design

Lonaepsomatropin has been evaluated in a comprehensive clinical development program, including three completed multinational phase 3 clinical trials in pediatric GHD. The phase 3 pivotal heiGht trial (NCT02781727) was a multi-center, randomized, open-label, active-controlled trial of lonaepsomatropin compared with somatotropin over 52 weeks in treatment-naïve, prepubertal children (aged 3–11 years for females, 3–12 years for males) with GHD (2). Lonaepsomatropin was administered as a once-weekly SC dose of 0.24 mg hGH/kg/week, while somatotropin was administered as a daily SC dose of 0.034 mg/kg/day (equivalent to 0.24 mg/kg/week). A total of 161 prepubertal children with GHD who met the eligibility criteria were randomly assigned in a 2:1 ratio to receive weekly lonaepsomatropin or daily somatotropin, respectively, for the 52-week treatment period.

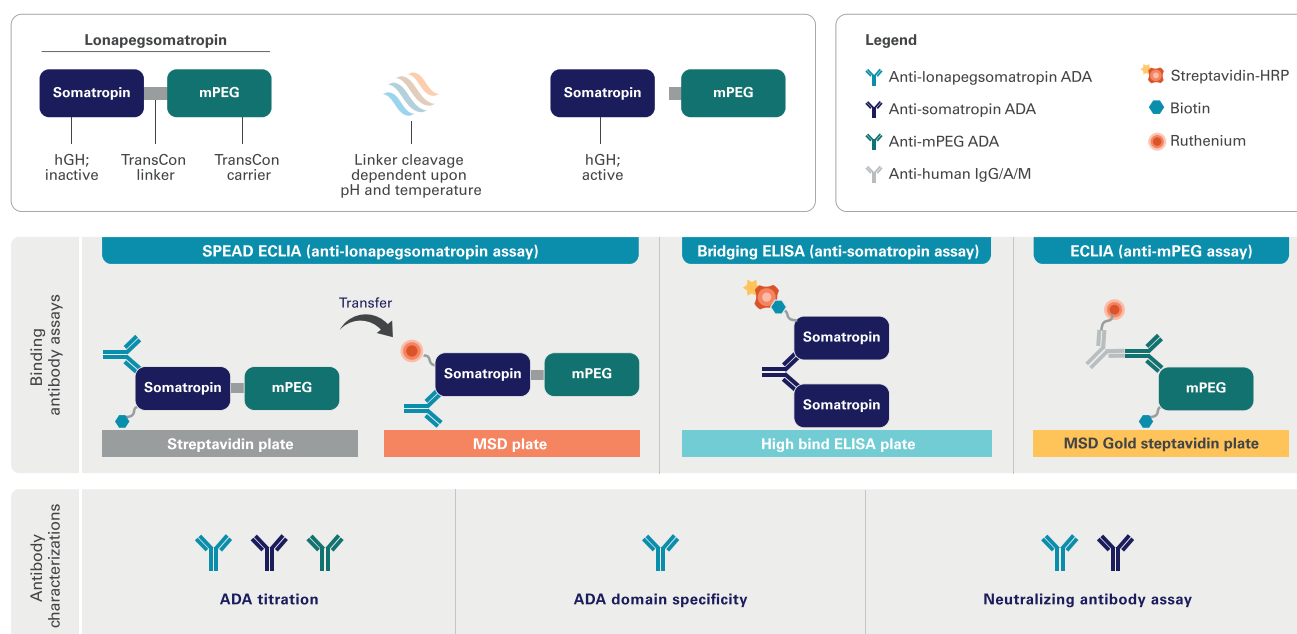


Fig. 1 Schematic diagram of the immunogenicity assays used to detect and characterize binding antibody responses towards lonaepsomatropin (intact prodrug), mPEG, and released hGH. Abbreviations: ADA, anti-drug antibody; ECLIA, electrochemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; hGH,

human growth hormone; HRP, horseradish peroxidase; IgG/A/M, immunoglobulin G/A/M; mPEG, methoxy polyethylene glycol; MSD, meso scale discovery; SPEAD, solid-phase extraction with acid dissociation

The phase 3 fliGHt trial (NCT03305016) was a multicenter, open-label trial of lonapegsomatropin in children 6 months to 17 years old, inclusive, with GHD (14). This trial included children (aged 3 to 17 years) who had been previously treated with commercially available daily somatropin (≥ 0.20 mg hGH/kg/week) for 13 to 130 weeks, and with no evidence of closed epiphyses. Additionally, children aged 6 months and up to 3 years old were either treatment-naïve or previously treated with daily somatropin (≥ 0.20 mg hGH/kg/week) for up to 130 weeks. A total of 146 children with GHD who met the eligibility criteria were enrolled to receive weekly SC lonapegsomatropin for 26 weeks. All children began lonapegsomatropin treatment at a dose of 0.24 mg hGH/kg/week, delivered via vial and syringe, regardless of prior daily somatropin use.

The phase 3 enliGHten trial (NCT03344458) was a multicenter, long-term, open-label extension trial to the heiGHt and fliGHt trials (5, 15). For most children, lonapegsomatropin was administered weekly by SC injections at approximately 0.24 mg hGH/kg/week, except in those who previously had their doses adjusted in the parent trial. The children from the heiGHt trial who were on daily somatropin switched to lonapegsomatropin upon enrollment into the enliGHten trial. Children received lonapegsomatropin for up to 260 weeks. A total of 298 children were enrolled, comprising a considerable proportion of individuals from the parent trials: 158 (98%) transitioned from the heiGHt trial, while 140 (96%) transitioned from the fliGHt trial (2, 14).

All clinical batches of lonapegsomatropin used in these trials met established product quality specifications, including predefined critical quality attributes such as host-cell protein levels, aggregates, and particles.

Samples for Immunogenicity Evaluation

Serum samples were collected for immunogenicity evaluation from 298 children throughout the three phase 3 trials of lonapegsomatropin in pediatric GHD. These samples were analyzed for ADAs against lonapegsomatropin (prodrug), somatropin (released), and mPEG (carrier). Samples were collected at baseline (before treatment) and at 13-week intervals throughout the duration of each trial, extending out to 312 weeks (6 years) of lonapegsomatropin treatment across the three trials. Additionally, an early sample was collected at week five in the initial phase 3 trial, heiGHt, which enrolled treatment-naïve children.

Immunogenicity Assays

Four dedicated immunogenicity assays, each including multiple tiers, were developed, qualified, and validated to characterize potential ADA response towards

lonapegsomatropin (intact prodrug), released somatropin (active drug), and mPEG (carrier) (Fig. 1). Assays were developed and validated based on current scientific practice published in White Papers (16–19) and regulatory guidance documents (20, 21). After an initial screening analysis in each of the 3 assays, samples with a result above screening cut point (CP) were confirmed through pre-incubation in a confirmatory buffer specific to the respective assay. Titrations of confirmed positive ADA samples were performed by serial dilution. Five different positive control antibodies were applied to characterize the assays and to ensure analytical performance during sample analysis. For the anti-lonapegsomatropin assay, a lonapegsomatropin-affinity purified rabbit polyclonal antibody (ADA-3; BioAgilytix) was employed. In the anti-somatropin assay, the antibodies used included a non-purified sheep polyclonal antibody (ADA-1; Biocheck 70332) and sensitivity estimate was evaluated by applying a somatropin-affinity purified goat polyclonal antibody (ADA-2; R&D Systems AF1067). The anti-methyl-polyethylene glycol (mPEG) assay utilized a rabbit monoclonal immunoglobulin G (IgG) antibody (ADA-4; clone RM105, GeneTex GTX60859), and an individual, non-purified, anti-mPEG positive human serum (ADA-5) was used as a positive control for mPEG antibody detection in human serum.

Treatment-induced ADAs were classified into two types. Treatment-emergent positive ADAs were defined as cases where the baseline (pre-treatment) sample was negative for ADAs, but the post-treatment sample was positive. When the baseline sample was positive for ADAs, and the post-treatment sample exhibited a titer at least four-fold greater than the baseline, this was defined as treatment-booster positive ADAs. ADA titers were assessed using serial dilution, and responses were considered low titer if reactivity was detected at ≤ 16 times the minimum required dilution (MRD) of the assay. Aligned with the white paper by Shankar *et al.*, positive ADA responses were defined as persistent when detected at ≥ 2 sampling time points, with a minimum interval of 16 weeks between the first and the last positive detections (22).

To calculate the incidence of ADAs, data from baseline (prior to initial lonapegsomatropin dose) through the end of the enliGHten trial were included. For children treated with lonapegsomatropin during the heiGHt or fliGHt trials, baseline was defined as the start of the heiGHt or fliGHt trials, and ADA data from both the heiGHt or fliGHt trial and the enliGHten trial were combined to determine ADA status. For children treated with somatropin in the heiGHt trial who initiated lonapegsomatropin treatment upon entering the enliGHten trial, only data collected during the extension trial were used for ADA analysis, with baseline defined as the last assessment prior to the first dose of lonapegsomatropin.

Anti-lona pegsomatropin Binding Antibody Assay

The anti-lona pegsomatropin binding assay was a solid-phase extraction with acid dissociation (SPEAD) immunoassay format where biotinylated lona pegsomatropin was bound to a streptavidin-coated plate and used to capture acid-dissociated antibody–drug complexes (Fig. 1). Dissociated antibodies were subsequently bound to a standard Meso Scale Discover (MSD) plate and then incubated with ruthenium-labeled lona pegsomatropin, which detected the bound anti-lona pegsomatropin antibodies that were proportional to the concentration of analyte detected (additional details can be found in supplementary information).

Anti-somatropin Binding Antibody Assay

The anti-somatropin binding antibody assay was a colorimetric bridging assay, utilizing somatropin to capture anti-somatropin antibodies from acid-treated samples in a 96-well plate (Fig. 1). Biotinylated somatropin was utilized to complete the bridging, while streptavidin–horseradish peroxidase (HRP) was used to generate an absorbance response that was proportional to the concentration of analyte detected (additional details can be found in supplementary information).

Anti-mPEG Binding Antibody Assay

The anti-mPEG binding antibody assay was an indirect electrochemiluminescent immunogenicity assay utilizing biotinylated mPEG to capture anti-mPEG antibodies from acid-treated samples in a 96-well plate (Fig. 1). A mixture of ruthenium-labeled (Ru-) anti-human IgG/A/M antibodies or Ru-labeled anti-rabbit IgG (to detect surrogate positive control rabbit anti-methoxy-PEG monoclonal antibody, ADA-4; clone RM105, GeneTex GTX60859) or human anti-methyl-polyethylene glycol positive serum (ADA-5), respectively, was utilized to detect the bound anti-mPEG antibodies proportional to the amount of analyte detected (additional details can be found in supplementary information).

Anti-somatropin neutralizing antibody assay

The anti-somatropin neutralizing antibody assay was cell-based, using the rat lymphoma cell line (Nb2-11) identical to the recommended cell line for *in vitro* cell-based bio-identity test for somatropin (USP 126). Samples confirmed positive in the anti-somatropin or anti-lona pegsomatropin binding antibody assays were analyzed in this

assay (additional details can be found in supplementary information).

Validation Parameters

Each method of validation included assay sensitivity, precision, the minimum required dilution (MRD), drug interference/tolerance, assay screening CP, confirmatory assay CP, intra- and inter-assay precision, robustness, selectivity (including evaluation using hemolyzed and lipemic serum), and sample stability (short-term handling, and freeze/thawing).

Results

Assay Validations and Characterization

Cut-point Determinations

A data-driven statistical approach was applied to establish analytical CPs during assay validation. Assay screening CPs were established to target 5% false positive samples (1% in the neutralizing antibody assay), whereas the confirmatory assay CPs were set to obtain a false positive rate of approximately 1%. The suitability of assay CPs applied in each trial was evaluated throughout the clinical development program. As trial-specific pre-treatment samples were collected, these initial assay CPs were evaluated and adjusted to ensure robust and accurate detection of ADAs across the three clinical trials. The details of the CPs specific to each assay and the resulting levels of false positive samples are outlined in Supplemental Table I.

Screening Assays

The screening assay performance was assessed based on screening CPs, screening sensitivity, and MRD for each binding antibody (Table 1). The screening CPs were 1.17 S/B, 1.17 S/B, and 3.05 S/B for the anti-lona pegsomatropin, anti-somatropin, and anti-mPEG binding antibody assays, respectively. The screening CP for the anti-somatropin neutralizing antibody assay was OSC – 0.008 AU (absorbance units) in the heiGHt and fliGHt trials and 0.765 S/B in the enliGHten trial. The relative sensitivity of the screening assays was 29 ng/mL for anti-lona pegsomatropin; 122 ng/mL (ADA-1) and 40 ng/mL (ADA-2) for anti-somatropin; 18 ng/mL for anti-mPEG binding antibodies; and 277 ng/mL (heiGHt and fliGHt) and 543 ng/mL (enliGHten) for anti-somatropin neutralizing antibodies. The MRD for the assays was determined to be 1:20, 1:25, 1:10, and 1:100 for anti-lona pegsomatropin, anti-somatropin, anti-mPEG, and anti-somatropin neutralizing antibodies, respectively.

Table I Overview of Key Assay Validation Parameters

	Minimum Required Dilution	Cut Points		Sensitivity		Total Precision ^b	Drug Tolerance
		Screening	Confirmatory	Screening	Confirmatory		
Anti-somatropin antibody binding assay	1:25	1.17 S/B	27% signal depletion	122 ng/mL (ADA-1) 40 ng/mL (ADA-2)	80 ng/mL (ADA-2)	CV < 12%	200 ng/mL somatropin (observed C _{max} = 86 ng/mL)
Anti-lonapegsomatropin antibody binding assay	1:20	1.17 S/B	18% signal depletion	29 ng/mL	39 ng/mL	CV < 9%	4,000 ng/mL lonapegsomatropin (somatropin equivalents) (observed C _{max} = 1230 ng/mL)
Anti-mPEG antibody binding assay	1:10	3.05 S/B	42% signal depletion	18 ng/mL (ADA-4)	5 ng/mL	CV < 19%	2,500 ng/mL mPEG (observed C _{max} = 13,100 ng/mL)
Anti-somatropin neutralizing antibody assay	1:100	OSC - 0.008 AU (heiGHt/fliGHt) 0.765 S/B ^a (enliGHten)	OSC - 0.083 AU (heiGHt/fliGHt) 0.782 S/B ^a (enliGHten)	277 ng/mL (heiGHt/fliGHt) 543 ng/mL (enliGHten)	Not applicable	CV < 18% (heiGHt/fliGHt) CV < 14% (enliGHten)	12.9 ng/mL somatropin (observed C _{max} = 86 ng/mL)

^aBackground defined as the OSC^bPrecision of low positive control

ADA, anti-drug antibody; AU, absorbance unit; CV, coefficient of variation; somatropin, recombinant human growth hormone; mPEG, methoxy polyethylene glycol; OSC, optimal stimulation control; S/B, signal/background

Confirmatory Assays

The performance of the confirmatory assays was evaluated based on confirmatory CPs, sensitivity, and total precision (Table I). Confirmatory CPs, based on signal depletion, were established at 18% for anti-lonapegsomatropin, 27% for anti-somatropin, and 42% for anti-mPEG assays. The relative sensitivity of the confirmatory assays was 39 ng/mL for anti-lonapegsomatropin; 80 ng/mL (ADA-2) for anti-somatropin; and 5 ng/mL for anti-mPEG binding antibodies. The total precision, expressed as %CV, was 21% for anti-lonapegsomatropin, 25% for anti-somatropin, 27% for anti-mPEG binding antibodies, and 30% for anti-somatropin neutralizing antibody assays.

Analytical Impact From Sample Hemolysis, Freeze/Thaws, and Extended Incubations

Impact from hemolysis on the sample material was evaluated by adding different concentrations of hemolyzed blood prior to analysis. Hemolysis levels up to 12.5% did not affect the detection of ADA in any of the three binding antibody assays. Antibody spiked, positive control samples were incubated at room temperature for at least 23 h for anti-lonapegsomatropin or anti-mPEG, at least 3 h for anti-somatropin, or processed through at least six repeated freeze/thaw cycles and produced reliable and uncompromised results. Similarly,

results were not significantly impaired in the cell-based anti-somatropin neutralizing antibody assay following the addition of 1% hemolysate or 6 freeze/thaw cycles.

Drug Tolerance in Immunogenicity Assays

The analytical sensitivity of the assay in the presence of drug was evaluated (Table I). Binding anti-lonapegsomatropin and anti-somatropin assays maintained an assay sensitivity of at least 100 ng/mL in the presence of clinically observed levels of lonapegsomatropin and somatropin (released from lonapegsomatropin during sample analysis). The sensitivity of the anti-mPEG antibody binding assay was slightly impaired at clinically relevant mPEG concentrations, but this had no impact on the evaluation of pre-existing anti-mPEG antibodies or the level of treatment-related antibodies observed. Potential interference from lonapegsomatropin (or somatropin) was minimized by analyzing samples at drug trough levels.

Assessment of Domain Specificity of Anti-Lonapegsomatropin Antibody Positive Samples

The anti-lonapegsomatropin antibody assay was characterized using serial dilutions of both anti-mPEG and anti-hGH positive control antibodies. The results demonstrate that the assay detected antibodies not only against

lonapegsomatropin, but also against mPEG and hGH with appropriate domain sensitivity (100 and 50 ng/mL, respectively).

An exploratory domain characterization tier was conducted to further assess the domain specificity in the samples positive for anti-lonapegsomatropin antibody. This analysis was performed using the validated anti-lonapegsomatropin antibody assay with two additional confirmatory tiers, in which the screening signal of all 31 confirmed anti-lonapegsomatropin-positive samples was depleted using somatropin or mPEG alone. The results, as shown in Supplemental Fig. 1, demonstrated that inhibition of the binding antibodies (detected in the anti-lonapegsomatropin screening assay) using somatropin alone was comparable to the inhibition using lonapegsomatropin, indicating that antibodies confirmed in the anti-lonapegsomatropin antibody assay were directed against the somatropin domain of the lonapegsomatropin prodrug. No signal inhibition was observed upon pre-incubation with mPEG alone.

Clinical Application

Summary of ADA

ADAs were assessed prior to initial lonapegsomatropin dose and every 13 weeks for 298 children treated with lonapegsomatropin across the heiGHt, fliGHt, and enliGHten trials. The mean duration of total treatment was 4.1 years, with a maximum of 6 years, across the three trials. Figure 2 illustrates the longitudinal frequency of the overall incidence of

ADAs (ie, combining the results across all three types of binding antibodies evaluated). Positive antibody responses were only transiently detected, primarily during the first year of exposure to lonapegsomatropin. With continued treatment, the incidence of children with positive antibodies declined over time. A total of twenty-five children (8.4%) exhibited detectable antibodies during lonapegsomatropin treatment, across any of the three antibody types evaluated (anti-lonapegsomatropin, anti-somatropin, and anti-mPEG). No more than 3% of the children were positive for ADAs at any time point.

Anti-lonapegsomatropin Binding Antibodies

Pre-existing anti-lonapegsomatropin binding antibodies were detected in 1/243 (0.4%) children (Table II). The incidence of treatment-emergent and treatment-boosted anti-lonapegsomatropin binding antibodies was 14/298 (4.7%) and 0/298 (0%), respectively.

Anti-somatropin Binding Antibodies

Pre-existing anti-somatropin antibodies were detected in 4/296 (1.4%) children prior to their first dose of lonapegsomatropin (Table II). Following initiation of treatment with lonapegsomatropin, a total of 16/298 (5.4%) children developed treatment-emergent anti-somatropin antibodies, and 1/298 (0.3%) participant developed a transient, treatment-related boost of a pre-existing anti-somatropin antibody level (fourfold increase in antibody titer).

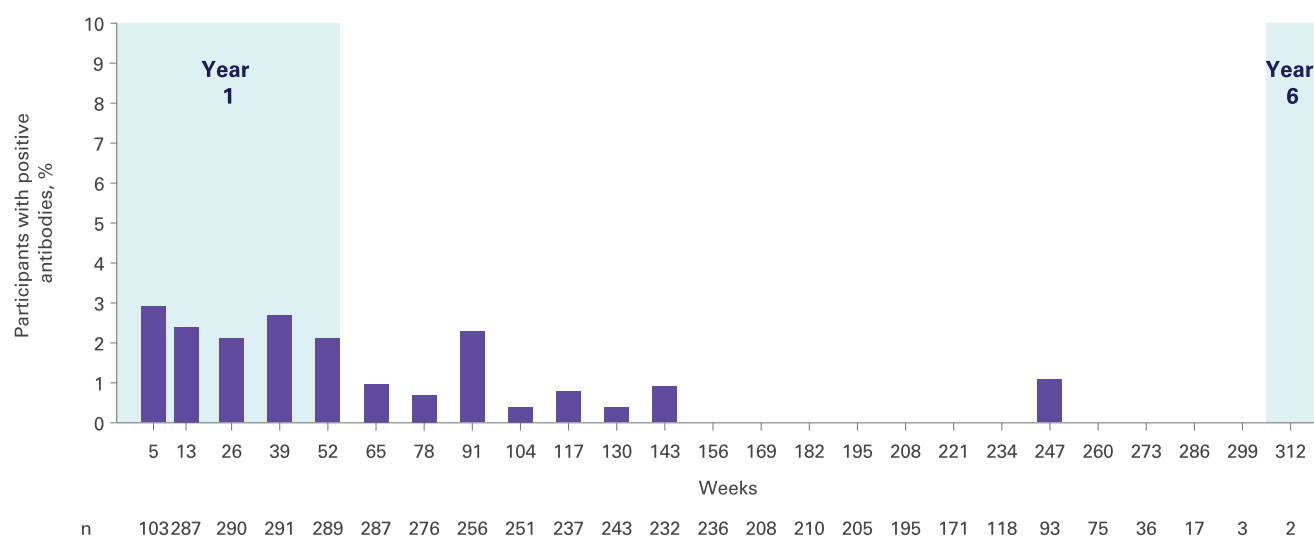


Fig. 2 Longitudinal incidence of combined ADAs throughout the duration of lonapegsomatropin treatment across the heiGHt, fliGHt, and enliGHten trials. The bar graph shows the percentage of children who tested positive for any of the three types of antibodies assessed: anti-hGH, anti-mPEG, and anti-lonapegsomatropin. The number of

participants with available assessments at each time point is indicated by n; the decrease in the number of children over time is mainly attributable to those who, according to protocol criteria, reached final or near-final height and completed GH treatment of pediatric GHD. At week 247, positive antibodies were detected in a single participant

Table II Incidence of Binding Antibodies Throughout the Duration of Lona pegsomatropin Treatment (Up to 6 Years) Across the heiGHt, fliGHt, and enliGHten Trials

	Anti-lona pegsomatropin	Anti-somatropin	Anti-mPEG
Pre-existing antibodies, ^a %	0.4	1.4	2.9
Incidence, treatment-emergent antibodies, ^b %	4.7	5.4	0.7
Incidence, treatment-booster antibodies, ^c %	0.0	0.3	0.0

The denominator (N) varies across groups due to differences in the total number of children included in each group at different points in the trial. These variations in (N) reflect factors such as missing data, participant exclusions, or other changes in the trial population

^aChildren with a positive binding antibody result at baseline prior to initial dose of lona pegsomatropin. Percentage calculated as n/N (anti-lona pegsomatropin: 1/243; anti-somatropin: 4/296; anti-mPEG: 7/243)

^bChildren with a positive binding antibody result at any visit post-dose and for whom no baseline antibodies were detected. Percentage calculated as n/N (anti-lona pegsomatropin: 14/298; anti-somatropin: 16/298; anti-mPEG: 2/298)

^cChildren with a positive binding antibody result at baseline and with $\geq$ fourfold increase in titer at any one visit post-baseline. Percentage calculated as n/N (anti-lona pegsomatropin: 0/298; anti-somatropin: 1/298; anti-mPEG: 0/298)

mPEG, methoxypolyethylene glycol

Anti-mPEG Binding Antibodies

Pre-existing anti-mPEG binding antibodies were detected in 7/243 (2.9%) children prior to their first dose of lona pegsomatropin (Table II). A treatment-emergent anti-mPEG antibody response was detected in 2/298 (0.7%) of the children treated with lona pegsomatropin, and no boosting of the pre-existing anti-mPEG antibodies was observed.

Anti-somatropin Neutralizing Antibodies

All samples with detectable antibodies against either lona pegsomatropin and/or somatropin were eligible for testing for neutralizing antibodies, as anti-lona pegsomatropin antibodies may recognize the somatropin moiety of the prodrug. None of the samples tested positive for neutralizing antibodies.

Antibody Responses Detected at ≥ 2 Time Points Spanning ≥ 16 Weeks

A total of 7 children (7/298, 2.3%) had treatment-emergent antibody responses to lona pegsomatropin that lasted beyond 16 weeks but resolved with continued lona pegsomatropin exposure. These children had varying treatment histories prior to initiating lona pegsomatropin. ADA levels declined in all children and became undetectable within 4 to 9 months of initial detection. An integrated evaluation of immunogenicity concluded that there were no signals of concern related to the antibody findings, as there were no significant effects on safety (previously reported in the enliGHten trial (15)), efficacy, or levels of insulin-like growth factor 1 (IGF-1, a biomarker of GH activity) (Supplemental Table II).

Discussion

Lona pegsomatropin, a prodrug of somatropin administered once weekly, is designed to provide sustained release of active, unmodified somatropin. As stipulated by regulatory guidance and white papers (16–21), a comprehensive immunogenicity assessment was undertaken to evaluate the immunogenic potential of lona pegsomatropin as a replacement therapy in children with GHD. In this study, a tiered immunogenicity testing strategy was implemented, encompassing 3 separately tiered antibody binding assays and a cell-based *in vitro* neutralizing antibody assay. The development and validation of these assays were important for ensuring reliable and adequate detection of ADA responses, minimizing variability while maintaining sensitivity to potentially clinically relevant ADA responses.

The lona pegsomatropin prodrug is a multi-domain product, consisting of a 4×10 kDa polymer (mPEG) transiently linked to somatropin via a proprietary TransCon linker. This transient conjugation is designed to temporarily shield the somatropin moiety prior to release, which has implications for immune recognition of both the carrier and the released hormone. Accordingly, comprehensive assessment of potential antibody responses across the different structural domains of lona pegsomatropin was important. Failure to evaluate immune reactivity of the different domains could lead to an incomplete understanding of the overall immune impact and potentially overlook specific antibody responses that may affect safety, pharmacokinetics, pharmacodynamics, or therapeutic efficacy (20).

The evaluation described in this paper suggests that lona pegsomatropin maintains an immunogenicity profile comparable to that of unmodified somatropin (23, 24) during long-term treatment. Using the TransCon technology

to release unmodified parent drugs has also resulted in low immunogenicity for both palopegteriparatide (TransCon PTH) and navepegritide (TransCon CNP) (25, 26). More broadly, among PEGylated biotherapeutics, PEGylation is known to modulate immune responses, in part through shielding of the parent molecule from immune cell recognition; however, anti-mPEG antibodies have nevertheless been reported following exposure to some PEGylated therapeutics (27, 28). This is the first comprehensive evaluation of anti-mPEG antibodies in a pediatric population with GHD. By developing and applying sensitive, robust assays, we found that the prevalence of anti-mPEG antibodies in children prior to treatment with lonapegsomatropin was low ($n = 7/243$; 2.9%), and the incidence of treatment-emergent anti-mPEG antibodies was even lower ($n = 2/298$; 0.7%). In contrast to our reporting on lonapegsomatropin, permanently PEGylated LAGH therapies have not reported on the potential impact of PEG-related antibodies (29), highlighting the rigor of our analytical approach and supporting the favorable long-term safety profile of lonapegsomatropin.

A treatment-related ADA response detected at ≥ 2 time points spanning ≥ 16 weeks was observed in 7 children. In 3 of 7 children, antibodies were detected in both the anti-lonapegsomatropin and anti-somatropin binding antibody assays, suggesting that the anti-lonapegsomatropin assay was solely detecting antibodies against the somatropin part of lonapegsomatropin. In support of this hypothesis, a domain characterization was carried out. Applying the anti-lonapegsomatropin antibody binding assay, two additional confirmatory tiers were performed, aiming at depleting the signal of all confirmed positive anti-lonapegsomatropin antibodies with either mPEG or somatropin alone. These results clearly demonstrate that antibodies confirmed in the anti-lonapegsomatropin assay were in fact antibodies directed against the somatropin domain of the lonapegsomatropin prodrug, as signals were depleted with somatropin alone.

The results of the immunogenicity assessment for lonapegsomatropin provided here are consistent with previously published phase 2 data for its predecessor molecule (30, 31). Furthermore, in adults with GHD treated with lonapegsomatropin for 38 weeks, the incidence of anti-lonapegsomatropin antibodies was low, and no neutralizing antibodies were detected (32). Overall, these findings indicate that lonapegsomatropin was well-tolerated and associated with a low likelihood of generating an antibody response. Consistent with this, the heiGHt and fliGHt trials demonstrated robust growth responses and favorable safety with lonapegsomatropin; long-term data from the enliGHten trial confirmed sustained efficacy and no new safety signals were identified (2, 5, 14, 15).

A limitation of the longitudinal height analyses is the progressive reduction in available assessments over time, primarily due to participants reaching adult height and

exiting the study upon completion of GH treatment, resulting in later timepoints being based on smaller sample sizes. Together, these results indicate that the low-titer, transient ADA responses observed did not adversely impact efficacy or safety outcomes.

Lastly, considering the low number of children with an ADA response to lonapegsomatropin, there are no indications suggesting discernible differences in the immunogenicity profile between children initiating GH therapy with lonapegsomatropin and those switching from daily somatropin to lonapegsomatropin therapy, underscoring the safety and efficacy of lonapegsomatropin therapy across children with various treatment journeys. These data suggest that transitioning from daily somatropin to weekly lonapegsomatropin therapy maintains the low risk of developing treatment-emergent antibodies.

Conclusion

This comprehensive immunogenicity evaluation applied a tiered, highly sensitive analytical approach to rigorously assess the immunogenicity of lonapegsomatropin in children with GHD, including those children with up to six years of treatment. The assays were robust and reproducible, able to detect ADAs against the intact prodrug, somatropin, and mPEG carrier across clinical trials. ADA responses were low titer, transient, and primarily observed within the first year of treatment, showing a long-term low immunogenicity risk of lonapegsomatropin, as seen for daily somatropin. The low immunogenicity observed may be attributable, in part, to the sustained release of unmodified somatropin following prodrug cleavage. These findings demonstrate both the clinical applicability of the assay strategy for complex long-acting protein therapeutics and that lonapegsomatropin exhibits an immunogenicity profile consistent with expectations for somatropin therapy.

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Data Availability The datasets generated and/or analyzed during the study are not publicly available but are available for non-commercial, academic purposes from the sponsor, absent legal reasons to the contrary, upon reasonable request. Further enquiries can be directed to ADS@ascendispharma.com.

Declarations

Conflict of interest PHM and VMB are employees of Ascendis Pharma A/S; AW is an employee of BioAgilytix Labs; CMF is an employee of Svar Life Science AB; ASK, MM, and ADS are employees of Ascendis Pharma, LLC.

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