Changes in Manufacturing Processes of Biologic Therapies Can Alter the Immunogenicity Profile of the Product

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Manufacturing process changes may alter the characteristics of a protein therapeutic. In 2009, somatropin (version 1.0), a recombinant human growth hormone therapeutic, underwent a manufacturing update (version 1.1). The immunogenicity of somatropin version 1.1 as a daily subcutaneous injection was evaluated in 2014 in a prospective, open-label, single-arm clinical study of treatment-naive pediatric patients with idiopathic human growth hormone deficiency for 1 year. The primary end point was the proportion of patients who developed antidrug antibodies (ADAs) after treatment. Eighty-two patients were enrolled. The mean (SD) treatment duration was 347 (53) days. The incidence of ADAs was 3.7%. No neutralizing antibodies were observed in the three patients with ADA-positive samples. Two patients (2.6%) had growth attenuation, but they were not ADA positive. The manufacturing changes for somatropin version 1.1 resulted in a similar safety and efficacy profile compared with somatropin version 1.0 and a different immunogenicity profile with a lower incidence of ADAs.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Clinical trials and postmarketing studies are currently used to establish biosimilarity to reference products and to assess immunogenicity after extensive manufacturing changes. Because the immunogenicity profile of a biologic cannot be predicted or assessed with analytical tests, clinical studies may be warranted to assess the cumulative impact of numerous manufacturing changes.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ After an updated manufacturing process, what was the immunogenicity profile of somatropin AQ version 1.1 daily s.c. injection at 1 year?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Manufacturing changes to biologics can result in a different immunogenicity profile with efficacy and safety comparable to the original product.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This study highlights the importance of evaluating immunogenicity in biologics that have undergone manufacturing changes to ensure that patients are deriving comparable efficacy and safety benefits.

Human growth hormone (HGH) is one of the most extensively studied pituitary hormones. The dominant circulating isoform is a single-chain peptide of 191 amino acids, also known as the 22K isoform of HGH. Biosynthetic 22K HGH was produced by Genentech, Inc., using recombinant DNA technology in the early 1980s.[1,2] For decades, humans have received pituitary-derived HGH and recombinant HGH (rHGH) for indications such as chronic renal insufficiency, Turner syndrome, Prader-Willi syndrome, idiopathic short stature, Noonan syndrome, short bowel syndrome, and severe growth hormone deficiency (GHD).[3] Due to concerns about transmitting human pathogens from pituitary-derived HGH, it was replaced by rHGH.[4]

Short stature in children, characterized by a height of ≥ 2 SDs below average, may be caused by inadequate endogenous secretion of HGH. GHD in children has multiple etiologies, including congenital or developmental defects during growth. Organic etiologies, such as central nervous system tumors, head trauma, radiation, and infection, may also factor into GHD. If no etiology is identified, the condition is labeled as idiopathic.[5] Physicians diagnose GHD using sensitive immunoassays to measure HGH in the blood. Children whose serum HGH levels do not increase substantially after administration of a growth hormone (GH) secretagogue, such as arginine, L-dopa, clonidine, or glucagon, or the induction of hypoglycemia are diagnosed with idiopathic GHD.[6]

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Many patients with idiopathic GHD release HGH after receiving a GH-releasing hormone, indicating that the fundamental abnormality is due to malfunction of the hypothalamus of inducing normal release.[6]

The recombinant form of HGH, somatropin, is the primary treatment for pediatric GHD, and over 25 different brands are available on the market. Somatropin injection for s.c. use is a lyophilized or liquid (AQ) form of rHGH produced by recombinant DNA technology, with an identical amino acid sequence to the 22K isoform of pituitary-derived GH. Somatropin is approved for various conditions associated with short stature, including idiopathic short stature, GHD, Turner syndrome, Noonan syndrome, *SHOX* deficiency, small size for gestational age without catch-up growth, and chronic kidney disease before renal transplantation.[7] Administration of somatropin should be optimized for each patient. For pediatric GHD, somatropin is administered as a weekly dose ≤ 0.3 mg/kg of body weight divided into daily s.c. injections.[7] Guidelines published by the Pediatric Endocrine Society recommend that HGH treatment at pediatric doses should stop when growth velocity falls below 2–2.5 cm per year.[8]

In 2009, the drug substance manufacturing process for somatropin was updated, using the same *Escherichia coli* GH cell line and an unchanged final formulation, to produce somatropin AQ version 1.1. This update was undertaken in order to remove animal-derived raw materials from the fermentation process, increase fermentation production titer, eliminate open processing, incorporate a streamlined purification process to reduce manufacturing complexity, and increase supply chain flexibility. Chemical and biologic characterization and potency data for somatropin AQ version 1.1 produced with the updated manufacturing process demonstrated comparability with somatropin AQ version 1.0 and somatropin AQ version 1.1. Somatropin AQ version 1.1 was approved by the US Food and Drug Administration (FDA) on May 15, 2014, and has been available on the US market since 2015.

Unwanted immune responses to protein therapeutics can result in the generation of antidrug antibodies (ADAs). Product-related factors that can arise from manufacturing process and influence immunogenicity include major modifications of the therapeutic protein (e.g., glycosylation or pegylation), protein or product aggregation, novel epitopes, degradation, oxidation, deamidation, and interactions between the proteins and excipients or impurities from the process or packaging.[9] Clinical consequences of immunogenicity may include loss of response, lack of efficacy, pharmacokinetic alterations, development of neutralizing antibodies, hypersensitivity, infusion reactions, and development of antibodies directed toward an endogenous protein, resulting in a severe adverse event (AE).[9,10] In a clinical study (used for the approval of somatropin AQ version 1.0) of 67 pediatric patients with GHD treated with somatropin AQ version 1.0, 15 patients (22.4%) developed ADAs after 12 months of treatment with no clinical sequelae (unpublished data). Because differences were known to exist between the manufacturing processes for somatropin AQ version 1.1 and somatropin AQ version 1.0, it was important to consider whether somatropin manufactured using the new process had a similar immunogenicity profile to that of somatropin from the original process.[10] Therefore, the immunogenicity of somatropin derived from the new process (somatropin AQ version 1.1) was evaluated in 2014 in a prospective, open-label, single-arm, clinical study of treatment-naive pediatric patients with GHD over 1 year (iStudy).

METHODS

Manufacturing process

The somatropin AQ version 1.1 process represented a comprehensive redesign of the somatropin drug substance manufacturing process (**Tables S1** and **S2**). Although the *Escherichia coli* cell line and cell banks were not changed, significant changes were made to the fermentation, harvest, purification, and bulk storage steps.

With fermentation, new automated equipment, media and nutrient feeds, and process parameter set points were used. These changes eliminated animal-derived raw materials, increased the rHGH titer, and reduced the number of fermentations needed to produce one bulk lot from three to one. During the harvest step, new homogenization equipment in a closed system was used to eliminate open processing steps, which previously exposed the product to the environment. The purification step incorporated new chromatography resin types to allow for fewer chromatography steps. For bulk storage, implementation of a freeze-thaw option for unformulated bulk provided greater supply-chain flexibility by allowing long-term storage prior to final formulation at the manufacturing site.

Clinical study to assess immunogenicity

Study design. This study was a phase IV, multicenter, open-label, single-arm trial of somatropin AQ version 1.1 in treatment-naive prepubertal children with GHD. Approximately 80 patients were planned to be enrolled from ~ 30 sites in the United States and to receive s.c. injections daily for 12 months of somatropin AQ version 1.1 per the prescribing information for somatropin AQ version 1.0.[7] Dose adjustments were allowed at month 6 for changes in weight and insulin growth factor-1 levels, if measured.

Screening was performed ≤ 28 days prior to day 1 unless otherwise specified, after which eligible patients began study treatment. Key inclusion criteria were age ≥ 3 and < 14 years, prepubertal status by physical examination, diagnosis of GHD by two standard pharmacologic tests obtained ≤ 12 months prior to enrollment, normal thyroid test results, and complete blood count. Key exclusion criteria were any previous rHGH treatment and any other short-stature etiologies. Patients who did not meet eligibility criteria could be rescreened once. Historical laboratory and radiographic tests were reviewed prior to obtaining informed consent/assent and initiating study treatment.

Patients who completed the month 12 visit were considered to have completed the study. AE information was collected by telephone at 28 ± 3 days after that visit. Patients who discontinued the study early were asked to return to the clinic 28 ± 3 days after the last dose for a visit to collect serum samples for GH levels and immunogenicity assessments.

The study was approved by the institutional review boards with jurisdiction over each study site. Signed parental permission was obtained prior to any study procedures. The study was registered at ClinicalTrials.gov (NCT02311894).

Objectives. The primary end point was to characterize the immunogenicity profile of somatropin AQ version 1.1 when administered as an s.c. injection for 12 months per the original prescribing information. As a key secondary end point, the clinical impact of immunogenicity was also assessed by evaluating patients for functional growth attenuation in association with the development of anti-GH antibodies (ADAs).

Study assessments. Assessments were scheduled at 3-month intervals following the baseline visit, with an additional visit for the month 1 blood draw, which was optionally done at the patient's home (**Table S3**).

Assessments included physical examination, height and weight measurements, assessment of pubertal status, and collection of serum samples for study-specific laboratory assessments (serum GH levels and immunogenicity assessments).

Growth response was monitored throughout the study; association of ADAs with changes in clinical response was evaluated. The annualized 6-month and 12-month height velocities for prepubertal patients were analyzed. Height velocities of patients who entered puberty during the study were excluded; however, these patients remained in the study, and their immunogenicity data were analyzed. Growth attenuation was defined as an initial growth response greater than the pretreatment height velocity followed by a reduction in growth response to below the pretreatment height velocity in the subsequent 6-month and 12-month treatment period or reaching ≤ 2 cm per year at any scheduled visit (month 3, 6, 9, or 12). Treated patients who entered puberty during the study were included in these analyses, and height velocity was assessed up to the last visit at which a patient was determined to be prepubertal.

Serum samples were obtained at baseline, month 1, month 3, month 6, month 9, and month 12 and evaluated for an ADA response to somatropin AQ version 1.1. All immunogenicity assessments and antibody assays were performed as previously described. The ADA assay was a radioimmunoprecipitation assay that used somatropin labeled with iodine^{125} to bind to and capture the anti-GH antibodies and also used polyethylene glycol solutions to precipitate heavy proteins, including ADAs. Detection of anti-GH antibodies was performed by measuring iodine^{125} on a gamma counter where normalized sample counts of iodine^{125} above the assay cut-point were considered anti-GH antibody positive. This same assay with the same cut-point was used for analyzing somatropin AQ version 1.0 ADAs and had consistent assay performance over time to allow for general comparisons of the new data to historical data. The neutralizing antibody (NAb) assay was a cell-based luminescence method developed specifically for this study. The method used an FDC-P1-F1 mouse cell line that expressed GH receptor. Cells were coincubated with somatropin and ADA-positive samples. Somatropin would bind to the cell GH receptor and release adenosine triphosphate, which was detected with CellTiter-Glo (Promega, Madison, WI) and measured using a luminescence plate reader. The presence of NAbs in the sample blocked somatropin from binding to the cell GH receptor and reduced or eliminated release of adenosine triphosphate. Samples below a normalized cut-point were considered NAb positive.

Immunogenicity data were described and interpreted using the industry best practices summarized by Shankar *et al.* in 2014.[11] This Association of American Physicians and Surgeons–endorsed white paper included definitions of evaluable and unevaluable patients, ADA incidence, treatment-induced and treatment-enhanced ADA, and transient and persistent ADA responses. All serum samples were analyzed for GH ADAs. All ADA-positive samples were then analyzed in a GH-NAb assay. Any ADA-positive samples with results ≥ 2.4 titer units were analyzed in a GH antibody binding capacity assay.

Statistical analyses. The target enrollment of 80 patients was chosen to allow for a projected 10–15% dropout rate and the exclusion of growth data for an estimated 10% of patients who might enter puberty during the study period, leaving sufficient patient numbers for comparison with previous studies of immunogenicity. With an evaluable sample size of 50 patients and an assumed 50% of patients developing anti-GH antibodies, the 95% confidence interval (CI) on the percentage of anti-GH antibody-positive patients was 35.53–64.47%.

Efficacy, immunogenicity, and safety data were summarized using descriptive statistics. Patient characteristics were summarized for the intent-to-treat (ITT) population, which included all enrolled patients. Efficacy and immunogenicity results were summarized for the modified ITT (mITT) population, which included all patients in the ITT population who received ≥ 1 dose of study drug and had ≥ 1 postbaseline follow-up assessment. Safety summaries were provided for the safety population, which included all enrolled patients who received ≥ 1 dose of study drug.

RESULTS

Patients

A total of 82 prepubertal patients from 22 sites in the United States who were naive to all rHGH therapies, including somatropin AQ, were enrolled. All enrolled patients (ITT population) received ≥ 1 dose of somatropin AQ version 1.1 and were included in the safety population. Of the 82 ITT patients, 1 patient did not have any postbaseline follow-up assessments and was therefore not included in the mITT population. Of the 82 patients enrolled, 78 (95.1%) completed 12 months of study therapy (**Table 1**). The mean duration of follow-up was 12.5 months (SD, 1.9 months) for the ITT population. Four patients (4.9%) discontinued study treatment due to AEs, loss to follow-up, or other reasons.

Most patients in the ITT population were boys (79.3%), white (84.1%), and not Hispanic or Latino (85.4%; **Table 2**). The mean age at enrollment was 9.0 years (SD, 1.9 years). All patients reported ≥ 1 previous and/or current medical condition, including GHD, asthenia, congenital jaw malformation, decreased appetite, dwarfism, food intolerance, selective immunoglobulin A immunodeficiency, and vitamin D deficiency. Sixty-two patients (75.6%) were receiving ≥ 1 concomitant medication during the study, with the most commonly reported medication classes being centrally acting sympathomimetics (26.8%), propionic acid derivatives (25.6%), and anilides (18.3%).

Table 1 Analysis populations and patient disposition

Characteristics, *n* (%)[a]	Total *N* = 82
Analysis populations	
ITT	82 (100)
Safety	82 (100)
mITT[b]	81 (98.8)
No postbaseline follow-up assessment	1 (1.2)
Patients who prematurely discontinued study medication	
No	78 (95.1)
Yes	4 (4.9)
Primary reason for discontinuation of study medication	
AE	1 (1.2)
Loss to follow-up	1 (1.2)
Withdrawal by patient	1 (1.2)
Other	1 (1.2)
Patients who completed the study	
Yes	78 (95.1)
No	4 (4.9)
Primary reason for discontinuing the study	
AE	1 (1.2)
Lost to follow-up	1 (1.2)
Other[c]	2 (2.4)

AE, adverse event; ITT, intent-to-treat population; mITT, modified intent-to-treat population.
[a]Percentages based on *N*. [b]One patient had no postbaseline follow-up data and was, therefore, excluded from the mITT population. [c]Included one patient who was discontinued in error and one patient who could not tolerate injections.

Table 2 Baseline demographics and clinical characteristics in the ITT population

Characteristic, *n* (%)[a]	Total *N* = 82
Age, mean (SD), years	9.0 (1.9)
Sex	
Male	65 (79.3)
Female	17 (20.7)
Ethnicity	
Not Hispanic or Latino	70 (85.4)
Hispanic or Latino	11 (13.4)
Not reported	1 (1.2)
Race[b]	
White	69 (84.1)
Asian	5 (6.1)
Black or African American	3 (3.7)
Other	3 (3.7)
Unknown	3 (3.7)
Weight, mean (SD), kg	23.9 (5.8)
Height, mean (SD), cm	121.7 (10.4)
Baseline height SDS, mean (SD)	−2.3 (0.5)
Baseline height SDS category	
≤−3 SD	12 (14.6)
≥−3 to ≤−2 SD	43 (52.4)
>−2 to ≤−1 SD	27 (32.9)
>−1 SD	0
Pretreatment height velocity, mean (SD), cm/year[c]	4.2 (2.1)

ITT, intent-to-treat; SDS, SD score.
[a]Percentages based on *N*. [b]Patients may have been counted in multiple race categories. [c]One patient had negative pretreatment height velocity because historical height (132.6 cm) was slightly greater than baseline height (131.37 cm); however, the site was closed, and data could not be updated.

Table 3 Time course of anti-GH antibodies in the mITT population

	Total *N* = 81
Patients positive for ADA at baseline, *n* (%)	0
Patients negative for ADA at baseline, *n* (%)	80 (98.8)
Patients with missing ADA at baseline, *n* (%)	1 (1.2)
Patients with postbaseline ADA results, *n* (%)	81 (100)
Patients positive for ADAs after initiating study medication, *n* (%)[a]	3 (3.7)
95% CI, %	0.8–10.4
Patients with positive titers by visit, *n* (%)[b]	
At baseline, *n* = 80	0
Month 1, *n* = 80	0
Month 3, *n* = 78	0
Month 6, *n* = 79	1 (1.3)
Month 9, *n* = 79	2 (2.5)
Month 12, *n* = 75	2 (2.7)
Median time to onset of ADA, months[c]	8.6

ADA, antidrug antibody; CI, confidence interval; GH, growth hormone; mITT, modified intent-to-treat.
[a]Percentage based on number of patients with postbaseline ADA assay result. Positive titer if titer ≥ 1.0. Unevaluable patients were excluded from the mITT population. [b]Positive titer if titer ≥ 1.0. Percentages based on *n*. [c]Median time (in months) between the first dose of study treatment and first instance of ADA in all patients with treatment-induced ADA.

Table 4 Analysis of ADA-positive patients

	Total *n* = 3
Patients NAb positive	0
Evaluation of ADA, *n* (%)	
Treatment induced[a]	3
Treatment enhanced[b]	0
Persistent[c]	3
Transient[d]	0
ADA titer range, minimum and maximum[e]	1.07–1.68

ADA, antidrug antibody; NAb, neutralizing antibody.
[a]A patient with negative or missing baseline ADA results and ≥ 1 positive postbaseline ADA result. [b]A patient with a positive ADA result at baseline who had ≥ 1 postbaseline titer results that were ≥ 0.60 titer units greater than the baseline titer result. [c]Treatment-induced ADA detected at ≥ 2 sampling time points during the treatment or follow-up period where the first and last ADA-positive samples (irrespective of any negative samples in between) were separated by a period of ≥ 16 weeks or where the last sampling time point was ADA positive. [d]Treatment-induced ADA detected at only 1 sampling time point during the treatment period or follow-up period (excluding the last sampling time point). [e]Includes all postbaseline titers for all patients with treatment-induced ADA.

Immunogenicity

In the mITT population, 81 patients were negative for anti-GH antibodies at baseline, and 3 patients developed ADAs after treatment with somatropin AQ version 1.1 (**Table 3**). One patient had a missing baseline level of anti-GH antibodies. The percentage of patients who developed ADAs was 3.7% (3 of 81 patients (95% CI 0.8–10.4%)). One patient was ADA-positive at months 6 and 9 (treatment was discontinued in error at month 9). Another patient was ADA-positive at months 9 and 12, and the third patient was ADA-positive only at month 12.

None of the three patients who developed ADAs were positive for NAbs (**Table 4**). All ADA responses were treatment induced. Furthermore, all ADA-positive samples had titers below the threshold for testing in the GH antibody binding capacity assay (see Study assessments section).

Secondary efficacy end points

A total of 78 patients (96.3%) in the mITT population had both baseline and postbaseline height velocity data and were analyzed for growth attenuation. Two patients (2.6%) exhibited growth attenuation during the study. However, neither patient had detectable ADAs at any of the postbaseline visits. One patient had an annualized height velocity of 8.0 cm per year at baseline (pretreatment height velocity), 9.5 cm at month 6, 7.7 cm at month 9, and 8.5 cm at month 12, indicating transient growth attenuation at month 9. The other patient had an annualized height velocity of 9.9 cm per year at baseline, 12.2 cm at month 3, 8.8 cm at month 6, 8.8 cm at month 9, and 9.0 at month 12, indicating growth attenuation at month 6. At all visits (including baseline), height velocities and height SD scores were similar between the group of patients who developed ADAs and those who did not.

Safety

The mean treatment duration was 347 days (SD, 53 days; range 45–378 days). Fifty patients had a dose modification at month 6,

primarily due to a change from baseline in weight (SD, 2 kg). Fifty-seven patients (69.5%) reported ≥ 1 AE during the study, most commonly headache (20.7%), vomiting (14.6%), and upper respiratory tract infection (9.8%; **Table S4**).

In the three patients who developed persistent treatment-induced ADA responses, no new AEs, including no anaphylactic reactions, occurred after the development of ADAs. Mild and moderate AEs were reported in 40.2% and 28.0% of patients, respectively. One patient experienced ≥ 1 grade 3 AE. An 11-year-old boy experienced a severe AE of idiopathic intracranial hypertension ~ 5 weeks after beginning study treatment that was considered related to somatropin. The study drug was withdrawn, and the patient's treatment was discontinued after a diagnosis of papilledema. Ten patients (12.2%) reported AEs that were considered related to study drug, most commonly headache (3.7%) and injection-site bruising (2.4%).

No serious AEs, AEs of special interest (AESIs), or deaths were reported. AESIs for this study included potential drug-induced liver injury and suspected transmission of an infectious agent via medicinal product. Ten patients (12.2%) experienced a drug-related AE, with one of these (1.2%) having a drug-related AE that led to withdrawal of study drug.

Eight patients (9.8%) experienced 11 AEs possibly indicative of hypersensitivity, which included rash (six patients), pruritus, and urticaria that were considered unrelated to somatropin AQ version 1.1. The events reported by the seven patients were considered unrelated to study treatment. In the remaining case, the rash resolved in 5 days without treatment. None of these patients developed ADAs. One patient each experienced injection-site erythema, injection-site pruritus, and injection-site reaction; all were considered related to somatropin AQ version 1.1, but all resolved within 5 days without treatment.

DISCUSSION

Somatropin has an extensive history of safety and efficacy in the treatment of GHD. To contextualize the current study results, we compared them with the results of a prior clinical study of 67 patients with GHD treated with somatropin AQ developed with the version 1.0 manufacturing process. This particular study was chosen as a benchmark due to the comparable patient demographics, treatment schedules, and ADA assessments and because it was the basis for the approval of somatropin AQ version 1.0. The previous study showed that 22.4% of patients developed ADAs by 12 months, but none had developed anti-GH antibodies with binding capacities ≥ 2 mg/L. ADAs were not linked to growth attenuation or other clinical sequelae. By comparison, in this current study, somatropin AQ version 1.1 was associated with a lower incidence of ADAs (3.7%) than somatropin AQ version 1.0, and at 6 months, no patients with GHD had developed antibodies with binding capacities ≥ 2 mg/L. All ADA responses were treatment-induced and persistent, and no growth attenuation was observed in patients who developed ADAs. Two patients met the study's definition of growth attenuation but were ADA negative at all time points. The anti-GH antibody responses were not neutralizing in any of the patients who developed ADAs. In the three patients who developed ADAs, no new AEs occurred after development of ADAs. Also of note, the observed ADA incidence rate was much lower than that seen in a previous pediatric study of somatropin AQ version 1.0 in GHD (observed ADA incidence of 3.7% (95% CI 0.8–10.4%) vs. the historically observed ADA incidence of 22.4% (95% CI 13.1–34.2%)).

One possible cause of the lower ADA incidence observed with somatropin AQ version 1.1 is the change to the purification process. Previous studies with HGH have shown the adjuvant effects of *Escherichia coli* protein (ECP) impurities on subsequent immunogenicity.[12] New chromatography resin types and modes of action used in the version 1.1 process may have led to differences in the types of immunoreactive ECP in the final version 1.1 product. Although product release test results of version 1.0 and version 1.1 showed comparably low levels of total ECP (< 30 ppm) as measured by enzyme-linked immunosorbent assay, future analysis of drug substance using orthogonal methods, such as mass spectrometry, could provide insight into the differences in the respective ECP populations between the processes. Such future work could help elucidate the potential impact of process changes on the immune response. The version 1.0 and version 1.1 products were also comparable in other product quality attributes that could be associated with immunogenicity, such as levels of aggregation as measured by high-performance size-exclusion chromatography, oxidation as measured by reversed-phase high-performance liquid chromatography, and deamidation as measured by high-performance ion-exchange chromatography. These product quality assay results for both version 1.0 and version 1.1 drug products were > 99.0% monomer by high-performance size-exclusion chromatography, > 99.0% main peak by reversed-phase high-performance liquid chromatography, and > 97.0% main peak by high-performance ion-exchange chromatography. Furthermore, stability studies showed comparable modes and rates of degradation between the two product versions.

No serious AEs or AESIs were reported in this study, and most AEs were mild to moderate in severity. Possible hypersensitivity events were reported in eight patients (9.8%), but none were ADA positive. In clinical studies of patients treated with somatropin AQ version 1.0 for various indications, none of the patients developed ADAs with binding capacities ≥ 2 mg/L. However, serious hypersensitivity reactions, including anaphylactic reactions and angioedema, have been reported in postmarketing use of somatropin AQ version 1.0.[7]

In studies with other biologics, formulation modifications yielded immunogenicity results similar to those from the original formulation. Treatment of patients with allergic asthma using a novel formulation of omalizumab in prefilled syringes resulted in a similar safety and immunogenicity profile, as was seen with the original lyophilized formulation of this anti-immunoglobulin E antibody.[13] However, cases have been documented where the presence of host cell impurities in biologics (discovered belatedly during clinical development) affected immunogenicity and required subsequent manufacturing updates to remove them.[12,14]

In 2018, ~ 340 biologics were licensed by the FDA for a variety of diseases, including oncology and immunology indications.[15] Now, the biologics marketplace is becoming populated with biosimilars. To date, 19 biosimilars have been approved by the FDA, with many more currently being reviewed (none are HGH products); these biosimilars have the potential to increase patient access to therapy due to their lower cost.[16,17] In rheumatoid arthritis, infliximab and its biosimilar SB2 exhibited similar immunogenicity profiles,

with reported ADA incidences of 14.6%, 14.9%, and 14.1% of patients in the infliximab/SB2, infliximab/infliximab, and SB2/SB2 groups, respectively, and comparable efficacy.[18] Thus, in addition to efficacy and safety data, clinical immunogenicity data are needed in order to assess the benefits and risks of not only new investigational medical products but also biosimilars.

CONCLUSIONS

Major changes to a manufacturing process may change the immunogenicity profile of a protein therapeutic. In the case of somatropin AQ version 1.1, the immunogenicity profile changed, and the incidence of ADAs was reduced (22.4% to 3.7%). Although a definitive cause of the improved profile was not identified, future identification of the types of low-level host cell proteins in the final drug product could elucidate the potential impact of process changes on the immune response. These data suggested that the impact on clinical outcomes in sensitive patient populations may need to be characterized and monitored to ensure patients derive comparable benefits. As biosimilars are introduced into the field and used as alternatives to the original biologics, their profiles should be monitored and characterized carefully.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Supplementary Material: Tables S1-S4.

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CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. D.M. designed the research. A.M. and R.O. performed the research. A.R., D.M., C.W., L.Y., M.V., V.Q., and C.A. analyzed the data.

DATA AVAILABLE STATEMENT

Qualified researchers may request access to individual patient level data through the clinical study data request platform (www.clinicalstudydatarequest.com). Further details on Roche's criteria for eligible studies are available here (https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Roche.aspx). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here (https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm).

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