Chapter 6

Clinical Relevance of Anti-exenatide Antibodies: Safety, Efficacy, and Cross-reactivity with Long-term Treatment

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ABSTRACT

AIMS Antibody formation to therapeutic peptides is common. This analysis characterizes the time-course and cross-reactivity of anti-exenatide antibodies and potential effects on efficacy and safety.

MATERIALS AND METHODS Data from ITT patients in 12 controlled (n=2225, 12-52wks) and 5 uncontrolled (n=1538, up to 3yrs) exenatide BID trials and 4 controlled (n=653, 24-30wks) exenatide once weekly (QW) trials with 1 uncontrolled period (n=128, 52wks) were analyzed.

RESULTS Mean titers peaked early (6-22wks), and subsequently declined. At 30wks, 36.7% of exenatide BID patients were antibody-positive; 31.7% exhibited low titers (≤125) and 5.0% had higher titers (≥625). Antibody incidence declined to 16.9% (1.4% higher titer) at 3yrs. Similarly, 56.8% of exenatide QW patients were antibody-positive (45.0% low/11.8% higher titer) at 24-30wks, declining to 45.4% positive (9.2% higher titer) at 52wks. Treatment-emergent anti-exenatide antibodies from a subset of patients tested did not cross-react with human GLP-1 or glucagon. Other than injection-site reactions, adverse event rates in antibody-positive and antibody-negative patients were similar. Efficacy was robust in both antibody-negative and antibody-positive patients (mean A1C: -1.0% and -0.9%, respectively, exenatide BID; -1.6% and -1.3% exenatide QW), with a similar range of A1C responses between antibody-negative and antibody-positive patients of any titer. In the small subset of higher-titer patients (5% exenatide BID; 12% exenatide QW), a wide range of A1C responses was observed, with a somewhat attenuated mean A1C reduction compared to antibody-negative patients.

CONCLUSIONS Antibody status was not predictive of individual safety or efficacy and these data do not support dose adjustment based on antibody response.

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INTRODUCTION

Exenatide is a 39-amino acid GLP-1 receptor agonist currently approved for twice-daily (BID) dosing as an adjunct to diet and exercise for glycemic control in patients with type 2 diabetes. An extended-release formulation, exenatide once weekly (QW), in which exenatide molecules are encapsulated into microspheres is under review by the FDA and has recently been approved in the EU. Following weekly subcutaneous injection, the microspheres slowly release exenatide to provide continuous plasma concentrations with minimal peak-to-trough fluctuation. Although the exenatide molecule is the same as that approved for BID administration, exenatide QW shows greater improvement in glycemic control due to continuous drug exposure with no increased risk of hypoglycemia in clinical trials.(1) Antibody formation to a therapeutic peptide is common, even when identical to an endogenous human peptide, as reported for exogenously administered human insulins.(2) As a synthetic peptide derived from a non-mammalian source, antibody formation in response to exenatide is expected.

Antibodies against a therapeutic peptide may elicit a wide range of consequences, from no detectable change to life-threatening conditions.(3-4) Of primary concern is altered drug safety or compromised efficacy. Antibody formation may elicit IgE-mediated hypersensitivity reactions ranging from local skin reactions to more severe systemic reactions such as anaphylaxis. Deleterious effects can also occur when antibodies against a therapeutic agent cross-react with endogenous proteins, in some cases neutralizing the endogenous protein. (5-6) Efficacy may also be attenuated (7-13), even to the extent that higher doses cannot overcome the clinical resistance induced by the antibody response.(14)

The objective of this posthoc analysis was to characterize the incidence of anti-exenatide antibodies and potential effects on efficacy and safety. Data were analyzed from over 3000 patients treated with exenatide (BID or QW) in clinical trials that had frequent assessments of antibody titers, glycemic outcomes, and safety parameters throughout treatment.

MATERIALS AND METHODS

Data

Data from Intent-to-Treat (ITT) patients with type 2 diabetes on backgrounds of diet and exercise, metformin, sulfonylurea, TZD or a combination of these agents in 12 placebo- or comparator-controlled (n=2225, 12-52 weeks) and 5 uncontrolled (n=1538, to 3 years) exenatide BID trials and 4 comparator-controlled (n=653, 24-30 weeks) exenatide QW trials with 1 uncontrolled period (n=128, 52 weeks) were analyzed. Entry criteria, background therapy, randomization, demographics, study design, and outcomes have been described previously.(1, 15-29)

Common clinical protocols were approved for each site by appropriate Institutional Review Boards. Patients provided written informed consent prior to participation. Studies were conducted in accordance with principles described in the Declaration of Helsinki (1946) up to and including the Seoul revision (2008).(30)

Assay Methods

Anti-exenatide antibodies in human sera were evaluated using a validated enzyme-linked immunosorbant assay (ELISA). The quasi-quantitative assay detects total antibodies (IgM, IgG, and IgA isotypes) specific to exenatide using excess antigen immobilized on a solid phase. Exenatide (5µg/mL in 0.05M carbonate buffer, pH9.5) was non-covalently bound to microtiter plates (Immulon2, Dynatech). Plates were incubated overnight to 3 days at 2-8ºC, washed with PBS/0.1%Tween20, and blocked for 1-2 hours (MegaBloc3, diluted 1:500, Cel Associates, Inc.). Samples were diluted 1:5 in either sample diluent (1%BSA/PBS/0.01%Tween20) or sample diluent containing exenatide (0.1mg/mL). After blocking, plates were washed; samples were added and incubated at room temperature for 1-2 hours. Following samples aspiration, plates were rinsed 3X with PBS/0.1%Tween20. Specific binding was detected with anti-mouse (positive controls) or anti-human Ig (negative controls and samples) conjugated to horseradish peroxidase for 1-2 hours. Plates were rinsed 3X with PBS/0.1%Tween20, then 2X with milli-Q water. Signal was generated with 50µL of o-phenylenediamine solution (Sigma) in 0.055M phosphate/0.024M citrate, pH5.0. After 10-15 minutes, the reaction was stopped with 50µL 4N sulfuric acid solution. Optical density was detected at 490nm. Data were analyzed with SoftMax software. During development of the exenatide clinical program, assays were conducted at Amylin Pharmaceuticals, Inc and Millipore Corp. (previously Linco); assay details may differ slightly between laboratories.

Antibody titers were determined by serial 1/5 dilutions after a minimal dilution of 1/25. Titer is expressed as the reciprocal of the highest dilution of sample that tests positive (i.e., a dilution of 1/125 is expressed as a titer of 125). Detection of positive antibody specimens is unaffected by exenatide concentrations up to 8ng/mL. The lower limit of assay sensitivity was established by statistical analysis of normal human serum samples using a "risk-based approach"(31-32), targeting a 5% false positive rate. Approximately 6% of samples from exenatide-naïve patients reported here tested positive (titer ≥25) for anti-exenatide antibodies, which may represent false positive signals or preexisting cross-reactive antibodies to endogenous peptides.

Cross-reactivity Assay

The assay described above was expanded to assess cross-reactivity of anti-exenatide antibodies to human GLP-1 and glucagon. Samples were diluted 1:5 in 5 different buffers: sample diluent alone, sample diluent containing exenatide (0.1mg/mL), GLP-1 (0.1mg/mL), glucagon (0.1mg/mL), or a nonspecific peptide (36-amino acids, 0.1mg/mL) with no sequence homology to exenatide, GLP-1 or glucagon.

For exenatide BID, samples from all antibody-positive (55 low, 39 higher titer) and 12 antibody-negative patients from a long-term study were tested for cross-reactivity with GLP-1 and glucagon.(33) Also, samples from all 116 higher-titer patients that were also

antibody-positive at last visit and 25 antibody-negative patients from 6 long-term studies were tested for cross-reactivity with GLP-1. Per protocol, the sample with the highest titer for each patient was evaluated. If a sample was cross-reactive, the entire profile for the patient would be evaluated. For exenatide QW, samples from all 36 higher-titer exenatide QW patients from a long-term study (baseline and highest titer observed) were assayed for cross-reactivity with GLP-1 and glucagon. If the patient was antibody-positive at endpoint or study termination, then that sample was also assayed.

Statistical Analysis

All analyses were performed using ITT populations (all randomized patients who received at least one exenatide injection).

A patient was defined as having treatment-emergent anti-exenatide antibodies if antibodies were present after the first exenatide injection following absence of antibodies or missing antibody measurement at baseline or if the titer increased by at least 3 dilutions from a detectable baseline measurement. Antibody incidence was summarized by treatment duration, titer, and titer group (low [≤125] or higher [≥625]). Geometric mean titers were summarized over time for observed data, with negative titers designated a value of 1. Mean (SE) change in A1C from baseline was summarized by antibody status and titer at last visit. Treatment-emergent adverse events (TEAEs; occurring during or after the first exenatide injection) were summarized by treatment. Potentially immune-related TEAEs were summarized by antibody status (negative or ever positive) at any visit. Statistical analysis was performed using SAS (9.2; SAS Institute, Inc., North Carolina).

Amylin Pharmaceuticals, Inc. and Eli Lilly and Company were study sponsors and were involved in study design, protocol development, and collection, review and analysis of data. Dr. Diamant had full access to data and contributed to data analysis, reviews, and decisions on content.

RESULTS

Antibody Incidence

To assess antibody incidence across 3 years of treatment, titers of anti-exenatide antibodies were examined in long-term uncontrolled exenatide BID trials (n=1538). Geometric mean titers peaked early in treatment (Weeks 6-16), and subsequently declined, with 39.4% and 65.2% reductions from peak at Weeks 30 and 52, respectively (Figure 1A). At Week 30, 36.7% of patients were antibody-positive (Figure 1B), with the majority (31.7% of all patients) exhibiting low antibody titers (≤125) and a minority (5.0%) exhibiting higher titers (≥625). Antibody incidence continued to decline over time, with 24.7% positive at Week 52, and 16.9% positive at 3 years.

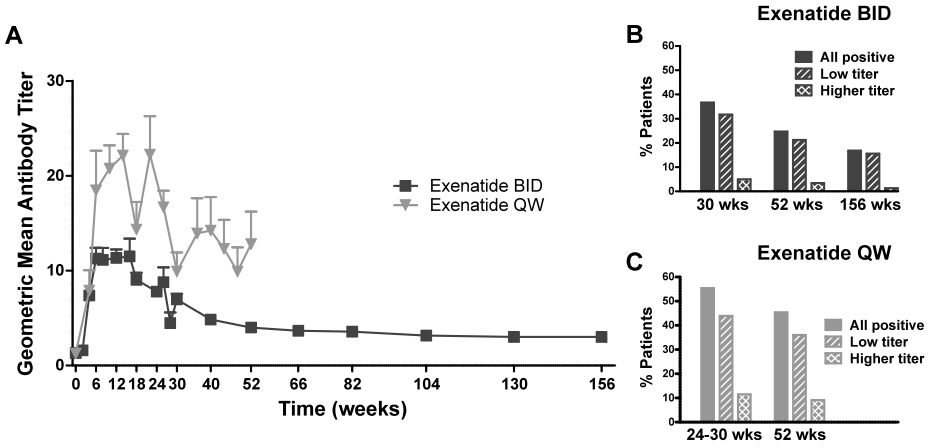

Figure 1 The incidence and titers of antibodies to exenatide peaked early in treatment and declined with continued therapy. A: Geometric mean (SE) antibody titers were assessed for Intent-to-Treat patients receiving exenatide BID in uncontrolled trials (n=1538, ITT) for up to 3 years, or exenatide QW in 4 controlled trials (n=653, ITT) through week 24-30, one of which continued on to an open-label extension period (n=128, ITT) to week 52. B: With exenatide BID treatment, the percentage of patients positive for antibodies to exenatide, as well as the percentage of patients with low (≤125) or higher antibody titers (≥625) was assessed at the indicated duration. At Week 30, 37% (242 of 660 patients with reportable titers) were antibody-positive, with the majority (32% of all patients) exhibiting low antibody titers (≤125) and a minority (5%) exhibiting higher titers (≥625). At Week 52, 25% (217 of 880 patients) were antibody positive (21% low/3% higher titer), and at 3 years, 17% (62 of 366 patients) were positive (16% low/1% higher titer). C: With exenatide QW treatment, at week 24-30, 57% (371 of 651 patients) were antibody-positive (45% low/12% higher titer). Antibody incidence continued to diminish over time, with 45% (54 of 119 patients) antibody-positive (36% low/9% higher titer) at week 52.

Similar trends were observed in controlled exenatide BID studies (n=2225), with 34.8% of patients antibody-positive (29.9% low/4.9% higher titer) at endpoint (12-52 weeks).

With exenatide QW, geometric mean titers peaked in a similar timeframe (Weeks 6-22) as exenatide BID (Figure 1A), and declined by approximately 50% between Weeks 24 and 52. At Weeks 24-30, 56.8% of patients were antibody-positive (45.0% low/11.8% higher titer, Figure 1C). At Week 52, 45.4% of patients were antibody-positive (36.1% low/9.2% higher titer).

Cross-reactivity With Human GLP-1 or Glucagon

Cross-reactivity to human GLP-1 was assessed in samples from 246 antibody-positive patients that received exenatide BID (n=210, 55 low, 155 higher titer) or QW (n=36 higher titer) in long-term trials. Cross-reactivity to GLP-1 was observed in only one low-titer exenatide BID patient. Importantly, when all samples for this patient were assayed, cross-reactivity to GLP-1 was observed in baseline samples (prior to exenatide administration), indicating a pre-existing reactivity. No adverse events consistent with an immune response were noted for this patient and antibody activity did not prevent a clinically significant improvement in glycemic control (40-week A1C change, -2.2%). These data indicate that this apparent antibody interaction with GLP-1 was unrelated to exenatide therapy and without any identifiable biologic consequence. Another patient had pre-existing reactivity with GLP-1 at baseline that was not detected in samples collected later in the study.

Cross-reactivity with human glucagon was assessed in samples from 130 patients that received exenatide BID (n=94, 55 low, 39 higher titer) or QW (n=36 higher titer) in long-term trials. No cross-reactivity was observed in any sample. These data indicate that treatment-emergent anti-exenatide antibodies do not cross-react with human GLP-1 or glucagon.

Safety

The incidence of TEAEs with exenatide BID and QW (independent of antibody status) was compared to placebo and active comparators to determine if exenatide was associated with a clinically meaningful increased incidence of TEAEs that may indicate an immune-related safety concern. In exenatide BID trials, the overall incidence rate of TEAEs was 82.6% for exenatide BID, 65.8% for placebo, 71.2% for TZD, and 70.9% for insulin. In exenatide QW trials, the incidence rate was 75.7% for exenatide QW, 71.5% for pioglitazone, 62.0% for sitagliptin, and 61.0% for insulin glargine. Exenatide treatment was not associated with increased TEAEs other than gastrointestinal events and injection-site reactions, both of which were generally mild and transient. The lack of increased safety signal (especially hypersensitivity reactions) with exenatide suggests that it is unlikely that anti-exenatide antibodies present an increased safety risk.

In addition, the incidence of potentially immune-related TEAEs (preferred terms provided in Table 1) was compared between exenatide-treated patients that were antibody-negative or ever antibody positive. With exenatide BID, antibody-positive patients had a higher incidence of potentially immune-related TEAEs (antibody positive, 10.9%; antibody negative, 5.6%; Table 2). The most notable difference was an increased incidence of injection-site pruritus (antibody positive, 1.4%; antibody negative, 0.1%).

With exenatide QW, a higher incidence of potentially-immune related TEAEs was observed in antibody-positive (22.4%, Table 2) versus antibody-negative (12.2%) patients. This was predominantly due to an increase in injection-site reactions that were typically mild, transient, and resolved without interruption of exenatide therapy. Importantly, no anaphylactic reactions were reported for either exenatide formulation.

Efficacy

Glycemic control (measured by A1C) was examined to assess whether anti-exenatide antibodies were associated with changes in efficacy. Analyses were conducted using ITT populations, thus data from all patients that received at least one dose of exenatide (including those that terminated early) were included. For exenatide BID patients in controlled trials (12-52 weeks), the mean A1C change from baseline was comparable between antibody-negative (-1.0%, 95%CI [-1.05,-0.92]) and antibody-positive patients (-0.9% [-1.00,-0.79], Figure 2A). Mean A1C reductions by titer at last visit (Figure 2B) were comparable between low-titer and antibody-negative patients (-1.0% for both). There was a trend towards reduced efficacy with increasing titer, resulting in a -0.5% mean A1C reduction for the 5% of patients with higher titers. Importantly, the range of A1C responses did not differ between higher-titer and antibody-negative patients, indicating that higher titers did not necessarily reduce efficacy. In fact, almost half of the higher-titer patients (49.3%) experienced A1C reductions of at least 0.5% (Figure 2B). Among the low percentage of exenatide BID patients who withdrew due to loss of glucose control (2% of all patients), half (1%) were antibody positive (0.3% higher titer) at last visit. Similar trends were observed with 3 years of exenatide BID in uncontrolled studies.

Table 1 Preferred terms (MedDRA version 12.0) of potentially immune-related adverse events

Allergic bronchitis	Hypersensitivity	Pruritus generalised
Allergic colitis	Idiopathic urticaria	Rash
Allergic cough	Immediate post-injection reaction	Rash erythematous
Allergic cystitis	Injection site dermatitis	Rash follicular
Allergic keratitis	Injection site eczema	Rash generalised
Allergic oedema	Injection site erythema	Rash macular
Allergic otitis media	Injection site hypersensitivity	Rash maculo-papular
Allergic pharyngitis	Injection site induration	Rash maculovesicular
Allergic respiratory symptom	Injection site inflammation	Rash papular
Alveolitis allergic	Injection site macule	Rash pruritic
Anaphylactic reaction	Injection site nodule	Rash pustular
Anaphylactic shock	Injection site oedema	Rash vesicular
Anaphylactoid reaction	Injection site papule	Reaction to drug excipients
Anaphylactoid shock	Injection site photosensitivity reaction	Reaction to preservatives
Angioedema	Injection site pruritus	Reversible airways obstruction
Arthralgia	Injection site pustule	Scleral oedema
Arthritis	Injection site rash	Scleritis allergic
Arthritis allergic	Injection site reaction	Skin oedema
Asthma	Injection site recall reaction	Small bowel angioedema
Auricular swelling	Injection site streaking	Stevens-Johnson syndrome
Bronchial hyperreactivity	Injection site swelling	Stridor
Bronchial oedema	Injection site urticaria	Suffocation feeling
Bronchospasm	Injection site vesicles	Swelling face
Circumoral oedema	Joint effusion	Swollen tongue
Conjunctival oedema	Joint swelling	Throat tightness
Corneal oedema	Laryngeal obstruction	Tongue oedema
Dermatitis allergic	Laryngeal oedema	Toxic epidermal necrolysis
Dermatitis	Laryngitis allergic	Toxic skin eruption
Dermographism	Laryngotracheal oedema	Tracheal obstruction
Diffuse cutaneous mastocytosis	Lip oedema	Tracheal oedema
Drug eruption	Lip swelling	Type I hypersensitivity
Drug hypersensitivity	Local swelling	Type II hypersensitivity
Drug rash with eosinophilia and systemic symptoms	Localised oedema	Type III immune complex mediated reaction
	Nasal oedema	
Encephalopathy allergic	Nephritis allergic	Type IV hypersensitivity reaction
Eosinophilia	Oculorespiratory syndrome	Urticaria
Eosinophilic oesophagitis	Oedema mouth	Urticaria cholinergic
Epiglottic oedema	Oedema mucosal	Urticaria chronic
Erythema multiforme	Oesophageal oedema	Urticaria contact
Erythema nodosum	Orbital oedema	Urticaria papular
Eye oedema	Oropharyngeal swelling	Urticaria physical
Eye swelling	Palatal oedema	Urticaria pigmentosa
Eyelid oedema	Periarthritis	Urticaria pressure
Face oedema	Periorbital oedema	Urticaria thermal
Gastrointestinal oedema	Pharyngeal oedema	Urticaria vesiculosa
Gingival oedema	Photosensitivity allergic reaction	Urticaria vibratory
Gingival swelling	Photosensitivity reaction	Visceral oedema
Haemorrhagic urticaria	Pruritus	Wheezing
Hereditary angioedema	Pruritus allergic	

Table 2 Potentially immune-related treatment-emergent adverse events that occurred with an incidence rate ≥5% by treatment-emergent antibody status

System Organ Class	Exenatide-Treated Patients			Comparators
	All Patients	Antibody negative	Antibody positive[b]	
Preferred Term[a]	n (%)	n (%)	n (%)	n (%)
Exenatide BID[c]				
N	2225	1273	952	1591
All potentially immune-related TEAEs	175 (7.9)	71 (5.6)	104 (10.9)	117 (7.4)
General disorders & administration site conditions	38 (1.6)	9 (0.7)	27 (2.8)	6 (0.4)
Injection site erythema	9 (0.4)	3 (0.2)	6 (0.6)	0 (0.0)
Injection site pruritus	14 (0.6)	1 (0.1)	13 (1.4)	2 (0.1)
Musculoskeletal & connective tissue disorders	63 (2.8)	30 (2.4)	33 (3.5)	49 (3.1)
Exenatide QW[d]				
N	653	189	464	554
All potentially immune-related TEAEs	127 (19.4)	23 (12.2)	104 (22.4)	54 (9.7)
General disorders & administration site conditions	86 (13.1)	9 (4.8)	77 (16.6)	17 (3.1)
Injection site erythema	26 (4.0)	1 (0.5)	25 (5.4)	5 (0.9)
Injection site pruritus	43 (6.6)	5 (2.6)	38 (8.2)	11 (2.0)
Musculoskeletal & connective tissue disorders	30 (4.6)	10 (5.3)	20 (4.3)	21 (3.8)

Abbreviations: TEAE, treatment-emergent adverse event.
a MedDRA Version 12.0
b Patients were considered antibody positive if a positive titer was detected at any visit.
c ITT patients with postbaseline antibody data that received 5 mcg or 10 mcg exenatide BID, or comparator (placebo, insulin or TZD) in 12 controlled studies
d ITT patients with postbaseline antibody data that received 2 mg exenatide QW or comparator (sitagliptin, pioglitazone, or insulin glargine) in 4 controlled studies

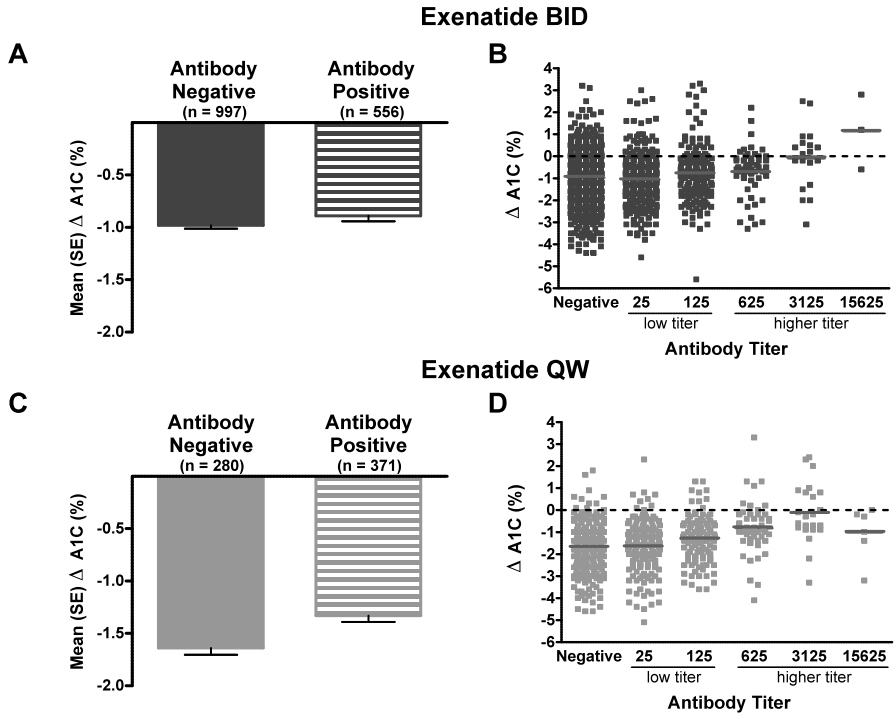

Figure 2 The change in A1C was comparable in antibody-negative and antibody-positive patients treated with exenatide BID or exenatide QW. A: Mean change (SE) in A1C at last visit (12-52 weeks of exenatide BID treatment) by antibody status (positive or negative) at last visit in exenatide BID controlled trials (Intent-to-Treat, n=2225, with 1553 patients with both antibody and A1C data at endpoint). B: Change in A1C at last visit by titer and titer group (low [≤125] or higher [≥625] titer) at last visit in exenatide BID controlled trials. C: Mean change (SE) in A1C at last visit (24-30 weeks of exenatide QW treatment) by antibody status at last visit in exenatide QW controlled trials (Intent-to-Treat, n=653, with 651 patients with both antibody and A1C data at last visit). D: Change in A1C at last visit by titer and titer group at last visit in exenatide QW controlled trials. Red bars indicate mean change in A1C.

Patients receiving exenatide QW experienced clinically relevant A1C reductions at endpoint regardless of antibody status (-1.6%, 95%CI [-1.77,-1.51] antibody negative; -1.3% [-1.45, -1.21] antibody positive, Figure 2C). As with exenatide BID, there was a trend towards reduced mean efficacy in the subset of patients (12%) with higher titers. The range of responses was generally consistent between antibody-negative patients and those of any titer, again indicating that higher titers do not necessarily result in reduced efficacy (Figure 2D). With exenatide QW, almost half of higher-titer patients (49.4%) exhibited A1C reductions of at least 0.8%. A low percentage (0.6%) of exenatide QW patients withdrew due to loss of glucose control; half had low antibody titers and half had higher titers at last visit.

DISCUSSION

Peptide therapeutics can induce an immune response, even when identical to the endogenous human peptide. An antibody response could partially attenuate or completely neutralize the efficacy of the drug. More importantly, an antibody response may have direct safety consequences (e.g. IgE-mediated hypersensitivity reactions, anaphylaxis, immune complex disease) or indirect safety consequences by neutralizing an endogenous system if the antibody cross-reacts with endogenous peptides. The latter could have lasting effects even after therapy is discontinued. Thus, it is crucial to characterize the immune response to any peptide or protein therapeutic.

Here we report an extensive analysis (over 3000 patients) that characterizes anti-exenatide antibodies in response to long-term exenatide treatment, including the time-course, and effects on safety (including cross-reactivity) and efficacy. Antibodies to exenatide developed early in treatment, however antibody incidence and titers declined over time. Importantly, this provides assurance that characterization of the immune response occurred when antibody titers were at their highest. Among antibody-positive patients, the majority exhibited low titers (≤125) and a small minority of patients exhibited higher titers (≥625). Other than an increased occurrence of injection-site reactions (typically mild in intensity, limited in duration, and no different than those observed in antibody-negative patients), antibody positivity did not alter the safety profile of exenatide. This is consistent with reports that anti-liraglutide antibodies are not associated with adverse events (34) but is in contrast to taspoglutide where rare but serious hypersensitivity reactions were reported and appeared to be associated with antibody formation.(35) Mean improvements in A1C with exenatide were comparable between antibody-negative and antibody-positive patients; however, a small percentage of patients (5% BID, 12% EQW) had higher-titer antibodies that trended towards reduced mean efficacy. Importantly, higher titers did not necessarily result in reduced response, as approximately half of the higher-titer patients had robust reductions in A1C. Thus, antibody titer is a poor predictor of A1C response.

A higher percentage of antibody-positive patients was observed with exenatide QW treatment than with exenatide BID; however, changes in safety and efficacy measures were consistent between formulations. The differences in immunogenicity between the formulations thus appear quantitative, not qualitative, in nature, consistent with presentation of the same exenatide molecule in the immediate-release and the extended-release formulations. The qualitative increase in the number of antibody-positive exenatide QW patients is likely due to continuous subcutaneous exenatide exposure and microsphere encapsulation.

Our findings are consistent with those from the LEAD-6 trial comparing exenatide BID with the injectable GLP-1 analog, liraglutide. For exenatide-treated patients, mean A1C reductions were attenuated in patients with high titers of anti-exenatide antibodies (-0.5%), compared to those with low titers (-1.0%) at Week 26.(34) The effect of anti-liraglutide antibodies on efficacy is less clear. For liraglutide-treated patients in LEAD-6, mean A1C reductions were attenuated in the small number of patients that were positive for antibodies to liraglutide (-0.5%, measured ≥5 days off drug at Week 78 (36) compared to -1.3% reported

for all liraglutide patients at Week 40 (37)). In other 26-week trials, mean A1C reductions in liraglutide(1.8 mg)-treated patients were similar between antibody positive (1.1%) and antibody negative (1.2%) patients.(34)

Treatment-emergent anti-exenatide antibodies did not cross-react with human GLP-1 or glucagon in vitro for the subset of antibody-positive patients tested. Samples from one low-titer patient cross-reacted with GLP-1; however cross-reactivity was observed in samples collected prior to exenatide administration, indicating a pre-existing immune reactivity. Cross-reactivity with GLP-1 was also detected in a baseline sample from another patient, however not in samples collected after exenatide exposure. In addition, epitope mapping experiments using pooled human polyclonal anti-exenatide antibodies determined that the primary epitope(s) for these antibodies are likely conformational, and require amino acid sequences from both the 15-23 and 29-39 regions for binding.(38) The observed lack of cross-reactivity may be explained by little to no homology between exenatide and GLP-1 or glucagon in these regions (Figure 3). These data indicate that anti-exenatide antibodies do not bind these endogenous gluco-regulatory hormones and are therefore unlikely to neutralize their activity.

									10									20									30										
Exenatide	H	G	E	G	T	F	T	S	D	L	S	K	Q	M	E	E	E	A	V	R	L	F	I	E	W	L	K	N	G	G	P	S	S	G	A	P	P	P	S
GLP-1 (7-36)	H	A	E	G	T	F	T	S	D	V	S	S	Y	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R								
Glucagon	H	S	Q	G	T	F	T	S	D	Y	S	K	Y	L	D	S	R	R	A	Q	D	F	V	Q	W	L	M	N	T									

Shading indicates common sequence.

Figure 3 Amino acid sequences of exenatide, GLP-1, and glucagon

These data contrast with those from the LEAD-6 trial, in which 4.4% (5 of 113) of samples positive for anti-exenatide antibodies cross reacted with GLP-1.(34) Detection assays with inherent differences in sensitivity, background, etc.(31) may underlie this difference, as an ELISA was used here and a radioimmunoprecipitation assay was used for LEAD-6. Preexisting cross reactivity may also explain the difference, as the only cross reactivity observed here was present prior to exenatide treatment. With no baseline data provided for LEAD-6, it is unclear if cross reactivity was preexisting or treatment-emergent.

A higher incidence of cross-reactivity with GLP-1 was reported for antibodies against liraglutide (55% [56 of 102] of antibody-positive patients from LEAD-1, -2, -3, -4, and -5 and 100% [4 of 4] in LEAD-6).(34) This cross-reactivity may result from high sequence homology between liraglutide and human GLP-1 and may be observed with other GLP-1 analogs. No information is available regarding cross-reactivity between antibodies to liraglutide and glucagon; however, given the close sequence homology between GLP-1 and glucagon (Figure 3), antibodies that cross-react with GLP-1 could also cross-react with glucagon. No information is available regarding cross-reactivity of anti-liraglutide antibodies with the main GLP-1 metabolite (9-36) or with the similar liraglutide metabolite.

Antibody formation to therapeutics may result in attenuated efficacy (7-12), such that dosage adjustment based on antibody status is necessary to maintain drug effect. In some cases, treatment is ineffective even at higher doses.(14) For exenatide, the range of A1C responses was consistent between antibody-negative patients and all titer groups of antibody-positive patients. Even in the small percentage of patients with the highest titers, approximately half experienced robust reductions in A1C. Given that only a small percentage of patients achieve an antibody titer that may affect response and the fact that antibody response is a poor predictor of individual efficacy, these data do not support the need for dosage adjustment based on antibody status.

A major strength of this report is the assay used to characterize the immune response to exenatide. When selecting an assay format to detect antibodies to a peptide therapeutic, specific characteristics such as sensitivity, background, isotypes detected, throughput, and interference by circulating drug concentrations should be considered.(31) To detect anti-exenatide antibodies, a direct ELISA assay was developed that was sensitive enough to detect low affinity antibodies and by dilution (titer) was able to discriminate between antibodies of no clinical relevance (low titer) and antibodies that may be associated with an attenuated response (higher titer). In addition, the assay was not sensitive to circulating drug concentrations and thus allowed for a full time course characterization of antibody response during exenatide treatment. With exenatide BID and QW treatment, maximum plasma exenatide concentrations are typically between 150 pg/mL and 350 pg/mL and assay validation demonstrated that concentrations up to 8 ng/mL would not interfere with antibody detection. Another strength is that the immune response to exenatide was characterized using ITT populations, thus all patients receiving exenatide were analyzed, including those that terminated early. This approach provides a conservative assessment of the consequences of antibody formation, as even patients that may have terminated early due to loss of glucose control are represented. An assessment of the low number of exenatide patients that withdrew due to loss of glucose control found no apparent association between antibody status and termination due to loss of efficacy.

A weakness of this report is the lack of a single clinical trial with a large number of patients receiving long-term exenatide therapy in which to characterize the immune response. However, the assessment presented here provides a full characterization of the antibody response to exenatide in over 3000 patients (some with up to 3 years of exposure) from multiple trials conducted worldwide. Another weakness is the lack of data available from the trials presented here regarding antibody formation in response to exenatide re-exposure in patients who had ceased exenatide therapy, however an independent trial demonstrated that antibody formation in response to exenatide re-exposure following a lapse of at least 2 months was not predictive of efficacy or safety, with no increased incidence of TEAEs or hypersensitivity reactions.(39)

The antibody response to exenatide was analyzed in a large number of patients to assess safety outcomes and potential neutralizing effects on the efficacy of exenatide. Although anti-exenatide antibodies were common, titers peaked early and declined thereafter. Importantly, there was no evidence that anti-exenatide antibodies negatively affected safety nor that these

antibodies could negatively affect the endogenous GLP-1 or glucagon systems. The antibody status of an individual treated with exenatide was a poor predictor of efficacy and thus these data do not support dose adjustment or a decision to suspend exenatide treatment based on antibody response per se. As with other antihyperglycemic agents, each patient's glycemic response should be monitored, and individualized clinical judgment made as to whether the patient is responding satisfactorily to exenatide treatment.

MF, BC, KFM and MT contributed to individual study designs. M.F. researched data, contributed to discussion, and reviewed/edited manuscript. K.F.M., M.D., T.D., B.C., and M.T. contributed to discussion and reviewed/edited manuscript. L.A.K. researched data and reviewed/edited manuscript. T.B.P. researched data, contributed to discussion, and wrote manuscript.

CONFLICTS OF INTEREST

MF, TD, BC, TBP, and LK are employees and shareholders of Amylin Pharmaceuticals Inc. MD is a consultant and speaker for Eli Lilly and Company, Novo Nordisk, and Merck, Sharp and Dohme, and a consultant for Sanofi-Aventis. Through MD the VU University Medical Centre in Amsterdam has received research grants from Amylin Pharmaceuticals Inc, Eli Lilly and Company, Novo Nordisk, Merck, Sharp and Dohme, Novartis, and Takeda. KM and MT are employees and shareholders of Eli Lilly and Company.

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