

Clinical relevance of anti-exenatide antibodies: safety, efficacy and cross-reactivity with long-term treatment

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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.

Methods: Data from intent-to-treat patients in 12 controlled (n = 2225, 12–52 weeks) and 5 uncontrolled (n = 1538, up to 3 years) exenatide twice-daily (BID) trials and 4 controlled (n = 653, 24–30 weeks) exenatide once weekly (QW) trials with 1 uncontrolled period (n = 128, 52 weeks) were analysed.

Results: Mean titres peaked early (6–22 weeks) and subsequently declined. At 30 weeks, 36.7% of exenatide BID patients were antibody-positive; 31.7% exhibited low titres (≤ 125) and 5.0% had higher titres (≥ 625). Antibody incidence declined to 16.9% (1.4% higher titre) at 3 years. Similarly, 56.8% of exenatide QW patients were antibody-positive (45.0% low/11.8% higher titre) at 24–30 weeks, declining to 45.4% positive (9.2% higher titre) at 52 weeks. 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 HbA1c change: -1.0 and -0.9% , respectively, exenatide BID; -1.6% and -1.3% exenatide QW). No correlation between change in HbA1c and titre was observed for exenatide BID, although mean reductions were attenuated in the small subset of patients (5%) with higher titres. A significant correlation was observed for exenatide QW with no difference between antibody-negative and low-titre patients, but an attenuated mean reduction in the subset of patients (12%) with higher titres.

Conclusions: Low-titre anti-exenatide antibodies were common with exenatide treatment (32% exenatide BID, 45% exenatide QW patients), but had no apparent effect on efficacy. Higher-titre antibodies were less common (5% exenatide BID, 12% exenatide QW) and within that titre group, increasing antibody titre was associated with reduced average efficacy that was statistically significant for exenatide QW. Other than injection-site reactions, anti-exenatide antibodies did not impact the safety of exenatide.

Keywords: exenatide, GLP-1, glycaemic control, incretin therapy, type 2 diabetes

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Introduction

Exenatide is a 39-amino acid glucagon-like peptide (GLP)-1 receptor agonist currently approved for twice-daily (BID) dosing as an adjunct to diet and exercise for glycaemic control in patients with type 2 diabetes. An extended-release formulation, exenatide once weekly (QW), in which exenatide molecules are encapsulated into microspheres has recently been approved. 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 glycaemic control as a result of continuous drug exposure with no increased risk of hypoglycaemia 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 immunoglobulin (Ig) E-mediated hypersensitivity reactions ranging from local skin reactions to more severe systemic reactions such as anaphylaxis. Deleterious effects

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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 *post hoc* analysis was to characterize the incidence of anti-exenatide antibodies and potential effects on efficacy and safety. Data were analysed from over 3000 patients treated with exenatide (BID or QW) in clinical trials that had frequent assessments of antibody titres, glycaemic 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, thiazolidinedione (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 analysed. 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 immunosorbent 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 (5 µg/ml in 0.05 M carbonate buffer, pH 9.5) was non-covalently bound to microtitre plates (Immulon2, Dynatech, Alexandria, VA, USA). Plates were incubated overnight to 3 days at 2–8 °C, washed with PBS/0.1% Tween 20 and blocked for 1–2 h (MegaBloc3, diluted 1 : 500, Cel Associates, Pearland, Texas, USA). Samples were diluted 1 : 5 in either sample diluent (1% BSA/PBS/0.01% Tween 20) or sample diluent containing exenatide (0.1 mg/ml). After blocking, plates were washed; samples were added and incubated at room temperature for 1–2 h. Following samples aspiration, plates were rinsed three times with PBS/0.1% Tween 20. Specific binding was detected with antimouse (positive controls) or antihuman Ig (negative controls and samples) conjugated to horseradish peroxidase for 1–2 h. Plates were rinsed three times with PBS/0.1% Tween 20, then two times with milli-Q water. Signal was generated with 50 µl of *O*-phenylenediamine solution (Sigma-Aldrich, St. Louis, MO, USA) in 0.055 M phosphate/0.024 M citrate, pH 5.0. After 10–15 min, the reaction was stopped with 50 µl 4 N sulfuric

acid solution. Optical density was detected at 490 nm. Data were analysed with SOFTMAX software (Molecular Devices Co., Sunnyvale, CA). During development of the exenatide clinical program, assays were conducted at Amylin Pharmaceuticals, Millipore Corp. (previously Linco) and Covance Laboratories; assay details may differ slightly between laboratories.

Antibody titres were determined by serial 1/5 dilutions after a minimal dilution of 1/25. Titre 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 titre of 125). Detection of positive antibody specimens is unaffected by exenatide concentrations up to 8 ng/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 (titre ≥ 25) for anti-exenatide antibodies, which may represent false-positive signals or pre-existing 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 five different buffers: sample diluent alone, sample diluent containing exenatide (0.1 mg/ml), GLP-1 (0.1 mg/ml), glucagon (0.1 mg/ml) or a non-specific peptide (36 amino acids, 0.1 mg/ml) with no sequence homology to exenatide, GLP-1 or glucagon.

For exenatide BID, samples from all antibody-positive (55 low, 39 higher titre) 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-titre patients who 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 titre 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-titre exenatide QW patients from a long-term study (baseline and highest titre 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 titre increased by at least 3 dilutions from a detectable baseline measurement. Antibody incidence was summarized by treatment duration, titre and titre group (low [≤ 125] or higher [≥ 625]). Geometric mean titres were summarized over time for observed data, with negative titres designated a value of 1. Mean (SE) change in

HbA1c from baseline was summarized by antibody status and titre 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. The relationship between the anti-exenatide antibody titre (a discrete variable) and the change in HbA1c (a continuous variable) was assessed using a non-parametric Spearman's correlation analysis. Statistical analysis was performed using SAS (9.2; SAS Institute, NC, USA).

Results

Antibody Incidence

To assess antibody incidence across 3 years of treatment, titres of anti-exenatide antibodies were examined in long-term uncontrolled exenatide BID trials ($n = 1538$). Geometric mean titres 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 titres (≤ 125) and a minority (5.0%) exhibiting higher titres (≥ 625). Antibody incidence continued to decline over time, with 24.7% positive at week 52 and 16.9% positive at 3 years.

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

With exenatide QW, geometric mean titres 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 titre, figure 1C). At week 52, 45.4% of patients were antibody-positive (36.1% low/9.2% higher titre).

Cross-reactivity With Human GLP-1 or Glucagon

Cross-reactivity to human GLP-1 was assessed in samples from 246 antibody-positive patients who received exenatide BID ($n = 210$, 55 low, 155 higher titre) or QW ($n = 36$ higher titre) in long-term trials. Cross-reactivity to GLP-1 was observed in only one low-titre 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 glycaemic control (40-week HbA1c 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 who received exenatide BID

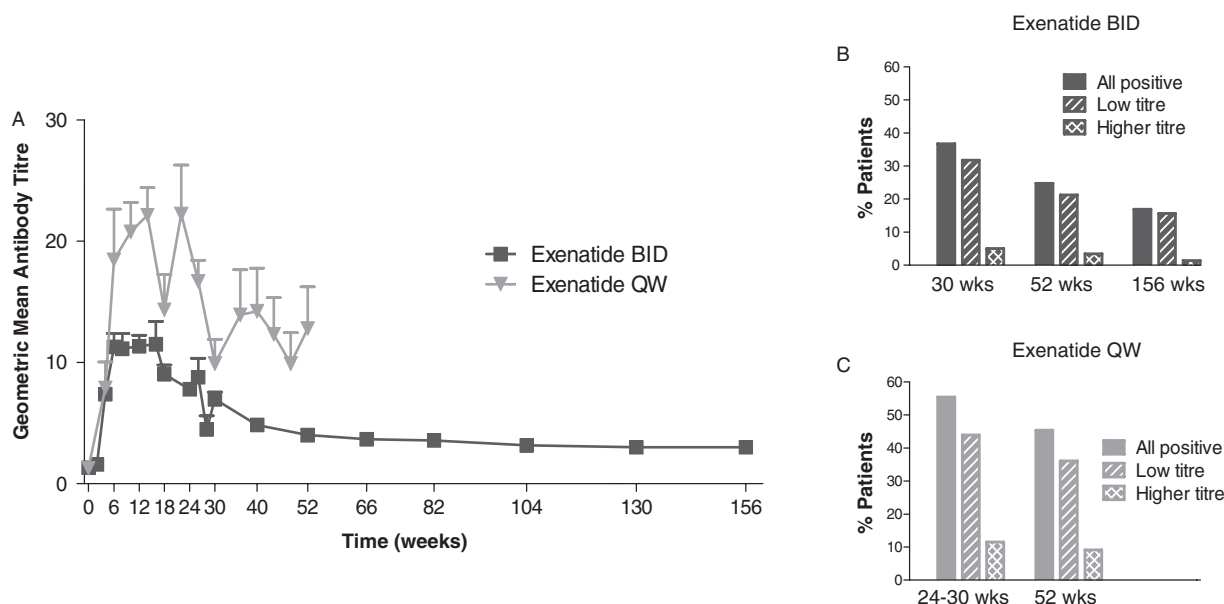


Figure 1. The incidence and titres of antibodies to exenatide peaked early in treatment and declined with continued therapy. (A) Geometric mean (SE) antibody titres were assessed for ITT patients receiving exenatide BID in uncontrolled trials ($n = 1538$, ITT) for up to 3 years or exenatide QW in 4 controlled trials ($n = 653$, ITT) through weeks 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 titres (≥ 625) was assessed at the indicated duration. At week 30, 37% (242 of 660 patients with reportable titres) were antibody-positive, with the majority (32% of all patients) exhibiting low antibody titres (≤ 125) and a minority (5%) exhibiting higher titres (≥ 625). At week 52, 25% (217 of 880 patients) were antibody-positive (21% low/3% higher titre) and at 3 years, 17% (62 of 366 patients) were positive (16% low/1% higher titre). (C) With exenatide QW treatment, at weeks 24–30, 57% (371 of 651 patients) were antibody-positive (45% low/12% higher titre). Antibody incidence continued to diminish over time, with 45% (54 of 119 patients) antibody-positive (36% low/9% higher titre) at week 52. ITT, intent-to-treat.

Table 1. Potentially immune-related TEAEs that occurred with an incidence rate $\geq 5\%$ by treatment-emergent antibody status.

System organ class Preferred term*	Exenatide-treated patients			Comparators n (%)
	All patients n (%)	Antibody-negative n (%)	Antibody-positive n (%)	
Exenatide BID†				
N	2225	1273	952	1591
All potentially immune-related TEAEs	175 (7.9)	71 (5.6)	104 (10.9)	117 (7.4)
General disorders and 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‡				
N	653	189	464	554
All potentially immune-related TEAEs	127 (19.4)	23 (12.2)	104 (22.4)	54 (9.7)
General disorders and 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)

TEAE, treatment-emergent adverse event.

*MEDDRA version 12.0 (Reston, VA, USA).

†ITT patients with postbaseline antibody data who received 5 µg or 10 µg exenatide BID or comparator (placebo, insulin or TZD) in 12 controlled studies.

‡ITT patients with postbaseline antibody data who received 2 mg exenatide QW or comparator (sitagliptin, pioglitazone or insulin glargine) in four controlled studies.

($n = 94$, 55 low, 39 higher titre) or QW ($n = 36$ higher titre) 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 (nausea and vomiting) and injection-site reactions. These adverse events peaked early in treatment (within the first 8–10 weeks) and decreased thereafter. They were typically mild, transient, decreased over time and had a generally low rate of reoccurrence for exenatide BID and QW regardless of antibody status (see figure S1, Supporting Information). 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 (list of 142 preferred terms, provided in Table S1, Supporting Information) was compared between exenatide-treated patients who 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 1) that was

predominantly because of an increase in injection-site reactions. These events were typically mild, transient and resolved without interruption of exenatide therapy regardless of antibody status.

With exenatide QW, a higher incidence of potentially immune-related TEAEs was observed in antibody-positive (22.4%, Table 1) versus antibody-negative (12.2%) patients, which was predominant because of an increase in injection-site reactions, the majority of which were mild, transient and resolved without interruption of exenatide therapy, regardless antibody status. Anti-exenatide antibodies did not extend the duration of injection-site adverse events with the majority of events resolving within 14 days in antibody-negative patients and within 7 days in antibody-positive patients. Importantly, no anaphylactic reactions were reported for either exenatide formulation.

Efficacy

Glycaemic control (measured by HbA1c) 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 who 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 HbA1c 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 HbA1c reductions by titre at last visit (figure 2B) were comparable between low-titre and antibody-negative patients (−1.0% for both). There was a trend towards reduced mean efficacy with increasing titre, resulting in a −0.5% mean HbA1c reduction for the 5% of patients with higher titres. Importantly, the range of HbA1c responses did not differ between higher-titre and antibody-negative patients, with

almost half of the higher-titre patients (49.3%) experiencing HbA1c reductions of at least 0.5%. No significant correlation between change in HbA1c and antibody titre was observed for exenatide BID ($r = 0.03761$, $p = 0.1385$). Among the low percentage of exenatide BID patients who withdrew because of loss of glucose control (2% of all patients), half (1%) were antibody-positive (0.3% higher titre) at last visit. Similar trends were observed with 3 years of exenatide BID in uncontrolled studies.

Patients receiving exenatide QW experienced clinically relevant HbA1c 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]. Mean HbA1c reductions by titre at last visit (figure 2D) were comparable between low-titre and antibody-negative patients (-1.5% low titre compared to -1.6% antibody-negative). A significant correlation between change in HbA1c and

antibody titre was observed for exenatide QW ($r = 0.22284$, $p < 0.0001$), with an attenuated mean response (-0.6%) for the 12% of patients with higher titres compared to low-titre and antibody-negative patients. Importantly, the range of responses was generally consistent between antibody-negative patients and those of any titre, with almost half of higher-titre patients (49.4%) exhibiting HbA1c reductions of at least 0.8%. A low percentage (0.6%) of exenatide QW patients withdrew because of loss of glucose control; half had low antibody titres and half had higher titres 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

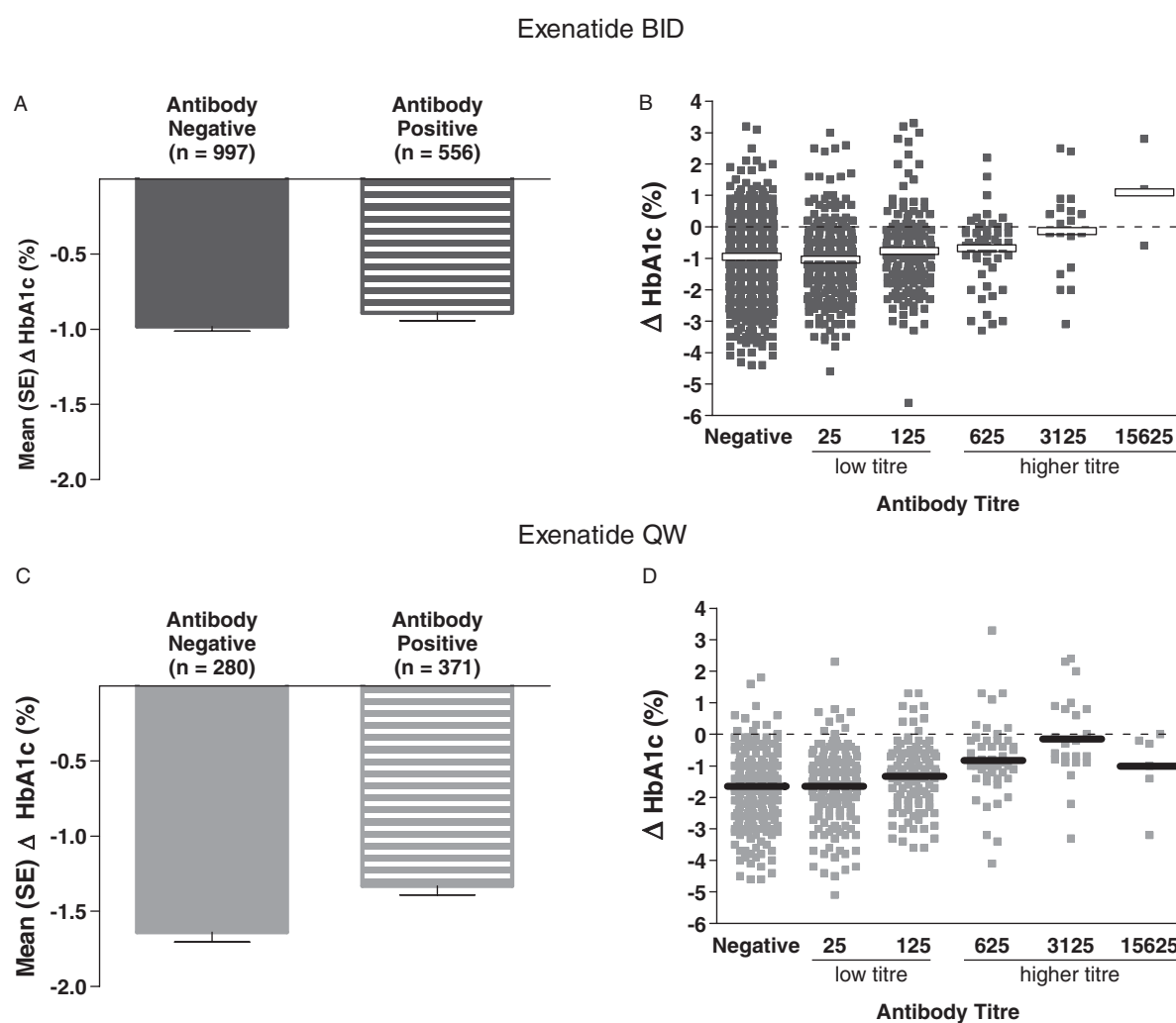


Figure 2. The change in HbA1c was comparable in antibody-negative and antibody-positive patients treated with exenatide BID or exenatide QW. (A) Mean change (SE) in HbA1c at last visit (12–52 weeks of exenatide BID treatment) by antibody status (positive or negative) at last visit in exenatide BID controlled trials (ITT, $n = 2225$, with 1553 patients with both antibody and HbA1c data at endpoint). (B) Change in HbA1c at last visit by titre and titre group [low (≤ 125) or higher (≥ 625) titre] at last visit in exenatide BID controlled trials. White bars indicate mean change in HbA1c. (C) Mean change (SE) in HbA1c at last visit (24–30 weeks of exenatide QW treatment) by antibody status at last visit in exenatide QW controlled trials (ITT, $n = 653$, with 651 patients with both antibody and HbA1c data at last visit). (D) Change in HbA1c at last visit by titre and titre group at last visit in exenatide QW controlled trials. Solid bars indicate mean change in HbA1c. ITT, intent-to-treat.

efficacy of the drug. More importantly, an antibody response may have direct safety consequences (e.g. IgE-mediated hypersensitivity reactions, anaphylaxis and 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 of antibody development, and effects on safety (including cross-reactivity) and efficacy. Antibodies to exenatide developed early in treatment; however, antibody incidence and titres declined over time. Importantly, this provides assurance that characterization of the immune response occurred when antibody titres were at their highest. Among antibody-positive patients, the majority exhibited low titres (≤ 125) and a small minority of patients exhibited higher titres (≥ 625). Other than an increased occurrence of injection site reactions [typically mild in intensity, limited in duration (≤ 14 days), 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 HbA1c with exenatide were comparable between antibody-negative and antibody-positive patients. The majority of antibody-positive patients (95% exenatide BID; 88% exenatide QW) had low-titre antibodies that did not impact the efficacy of exenatide. A small percentage of patients (5% exenatide BID, 12% exenatide QW) had higher-titre antibodies, and within that titre group, increasing antibody titre was associated with reduced mean efficacy. For exenatide QW, this association was statistically significant. While 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, for exenatide, the range of HbA1c responses was consistent between antibody-negative patients and the majority of antibody-positive patients. Even in the small percentage of patients with the highest titres, approximately half experienced robust reductions in HbA1c. Given that only a small percentage of patients achieve an antibody titre 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 higher percentage of antibody-positive patients was observed with exenatide QW treatment than with exenatide BID; however, changes in safety and efficacy measures were similar 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 formulation. The increase in the number of antibody-positive exenatide QW patients is probably because of continuous subcutaneous exenatide exposure and microsphere encapsulation.

Similar findings were reported for antibodies against another GLP-1 receptor agonist, liraglutide. Among patients treated with liraglutide (1.2 or 1.8 mg) for 26 weeks in LEAD trials, 80 of 951 (8.4%) patients were positive for anti-liraglutide antibodies [34]. The incidence of anti-liraglutide antibodies was lower than that reported for anti-exenatide antibodies among patients treated with exenatide BID in the LEAD-6 trial (61% at week 26) [34] and the exenatide BID results reported here (37% at week 30). A precise quantitative comparison of antibody incidence should be carried out with caution however, as antibody detection assays to different compounds will have different assay sensitivities, even if the same assay format is used [31]. Unfortunately, there is not a standardized method of normalizing sensitivity between drug antibody assays, as the lower limit of antibody detection is dependent on the affinity of the positive control to the drug in question. Patients positive for anti-liraglutide antibodies reported injection-site reactions, but with no serious or systemic immunologic adverse events. Consistent with results reported here for anti-exenatide antibodies, no difference in the change in HbA1c was reported between patients positive (-1.3%) or negative (-1.2%) for anti-liraglutide antibodies [34]. In the LEAD-6 trial, HbA1c reductions from baseline to week 78 in the four patients who were positive for antibodies to liraglutide (≥ 5 days off drug) were 0.4, 0.8, -1.4 and -1.9% , respectively [36], suggesting a variable efficacy response in the few patients positive for anti-liraglutide antibodies as is observed in patients with higher-titre anti-exenatide antibodies.

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-titre 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 probably conformational and require amino acid sequences from both the 15–23 and 29–39 regions for binding [37]. The observed lack of cross-reactivity may be explained by little to no homology between exenatide and GLP-1 or glucagon in these regions (see figure S2, Supporting Information). These data indicate that anti-exenatide antibodies do not bind these endogenous gluco-regulatory hormones and are therefore unlikely to neutralize their activity.

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]. This difference may be because of the detection assays employed, as an ELISA was used here and a radioimmunoassay was used for LEAD-6. Pre-existing 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 pre-existing or treatment-emergent.

A higher incidence of cross-reactivity with GLP-1 was reported for antibodies against liraglutide (56 of 102 antibody-positive patients from LEAD-1, -2, -3, -4 and -5 and 4 of 4 patients in LEAD-6) compared to anti-exenatide antibodies [34]. The differences in GLP-1 cross-reactivity between anti-exenatide and anti-liraglutide antibodies are probably because of differences in sequence homology compared to GLP-1. Exenatide shares less sequence homology with GLP-1 (53%) [38] than liraglutide does (97%) [39], which may underlie the higher incidence of antibodies against exenatide, as well as the lack of cross-reactivity with GLP-1 and the sequence-related hormone glucagon. No information is available regarding cross-reactivity between antibodies to liraglutide and glucagon; however, given the close sequence homology between GLP-1 and glucagon (see Supporting Information), antibodies that cross-react with GLP-1 may also cross-react with glucagon.

In the LEAD-6 study, patients originally randomized to exenatide were crossed over to liraglutide after 26 weeks of treatment. Interestingly, the 17 (6.7%) patients with the highest anti-exenatide antibody responses had, on average, a greater response to liraglutide than did low-titre and antibody-negative patients. Taken together with the GLP-1 cross-reactivity data, this observation suggests that higher-titre anti-exenatide antibodies do not cross-react with GLP-1 or liraglutide and thus previous exenatide treatment for this small group of higher-titre subjects should not negatively affect the efficacy of other compounds in the class. Data is not currently available on whether higher-titre antibodies to liraglutide might have carryover effects on the efficacy of other GLP-1 based therapies, but based on their cross-reactivity with GLP-1, it should be explored.

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 (titre) was able to determine that the majority of anti-exenatide antibody responses have no clinical relevance (low titre) while a small fraction are associated with an attenuated response (approximately half of higher-titre responses). 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 treatments, maximum plasma exenatide concentrations are typically between 150 and 350 pg/ml and assay validation showed 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 analysed, including those who terminated early. This approach provides a conservative assessment of the consequences of antibody formation, as even patients who may have terminated early because of loss of glucose control are represented. An assessment of the low number of exenatide patients who withdrew because of loss of glucose control

found no apparent association between antibody status and termination because of 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 showed 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 [40].

The antibody response to exenatide was analysed 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, titres 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 majority of antibody-positive patients had low-titre antibodies that did not impact the efficacy of exenatide. A small percentage of patients had higher-titre antibodies, and within that titre group, increasing antibody titre was associated with reduced average efficacy that was statistically significant for exenatide QW. However, as higher-titre antibodies did not necessarily result in lower individual glycaemic responses, the antibody status of an individual is not clinically useful for making treatment decisions. As with other antihyperglycaemic agents, each patient's glycaemic response should be monitored, and individualized clinical judgment made as to whether the patient is responding satisfactorily to exenatide treatment.

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Conflict of Interest

Amylin Pharmaceuticals and Eli Lilly and Company were study sponsors and were involved in study design, protocol development and collection, review, and analysis of data.

M.S.F. was an employee and shareholder of Amylin Pharmaceuticals, Inc. at the time this manuscript was prepared,

and was involved in study design, protocol development, collection, review and analysis of data, reviews and decisions on content. K. F. M. and M. E. T. are employees and shareholders of Eli Lilly and Company and were involved in study design, protocol development, collection, review and analysis of data, reviews, and decisions on content. M. D. is a consultant and speaker for Eli Lilly and Company, Novo Nordisk and Merck, Sharp and Dohme and a consultant for Sanofi-Aventis, and had full access to data and contributed to data analysis, reviews and decisions on content. Through M. D., 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. M.D. had full access to the data and contributed to data analysis, reviews, and decisions on content. T.D. was an employee and shareholder of Amylin Pharmaceuticals, Inc. at the time this manuscript was prepared, and contributed to data analysis, reviews, and decisions on content. B. B. C., T. K. B. P. and L. A. K. are employees and shareholders of Amylin Pharmaceuticals, Inc. B.C.C. contributed to discussion and reviewed/edited manuscript. L.A.K. researched and analyzed data and reviewed/edited manuscript. TBP researched and analyzed data, contributed to discussion and wrote the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Duration of frequent adverse events by antibody status. Top figure: The percentage of patients experiencing injection-site reactions (including injection-site pruritus, erythema, urticaria and rash), nausea and vomiting was assessed at 2-week intervals from baseline to endpoint by antibody status [antibody-negative (N = 1273) or ever antibody-positive (N = 952)] for patients receiving exenatide BID. Bottom figure: The percentage of patients experiencing injection-site reactions, nausea and vomiting was assessed at 2-week intervals from baseline to endpoint by antibody status [antibody-negative (N = 154) or ever antibody-positive (N = 499)] for patients receiving exenatide QW.

Figure S2. Amino acid sequences of exenatide, GLP-1 and glucagon.

Table S1. Preferred terms (MedDRA version 12.0) of potentially immune-related adverse events.

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