Treatment of Recent-Onset Type 1 Diabetic Patients With DiaPep277: Results of a Double-Blind, Placebo-Controlled, Randomized Phase 3 Trial

*Itamar Raz,[1] Anette G. Ziegler,[2] Thomas Linn,[3] Guntram Schernthaner,[4] Francois Bonnici,[5] Larry A. Distiller,[6] Carla Giordano,[7] Francesco Giorgino,[8] Liat de Vries,[9] Didac Mauricio,[10] Vlastimil Procházka,[11] Julio Wainstein,[12] Dana Elias,[13] Ann Avron,[13] Merana Tamir,[13] Rachel Eren,[13] Dana Peled,[13] Shlomo Dagan,[13] Irun R. Cohen,[14] Paolo Pozzilli,[15,16] and the DIA-AID 1 Writing Group**

OBJECTIVE

To evaluate safety and efficacy of DiaPep277 in preserving β-cell function in type 1 diabetic patients.

RESEARCH DESIGN AND METHODS

DIA-AID 1 is a multinational, phase 3, balanced-randomized, double-blind, placebo-controlled, parallel-group clinical study. Newly diagnosed patients (*N* = 457, aged 16–45 years) were randomized to subcutaneous injections of DiaPep277 or placebo quarterly for 2 years. The primary efficacy end point was the change from baseline in the area under the glucagon-stimulated C-peptide curve. Secondary end points were the change from baseline in mixed-meal stimulated C-peptide secretion and in fasting C-peptide and achieving target HbA_{1c} ≤7% (≤53 mmol/mol). Partial remission (target HbA_{1c} on insulin ≤0.5 units/kg/day) and hypoglycemic event rate were exploratory end points.

RESULTS

DiaPep277 was safe and well tolerated. Significant preservation of C-peptide secretion was observed in the DiaPep277-treated group compared with the placebo (relative treatment effects of 23.4%, *P* = 0.037, and 29.2%, *P* = 0.011, in the modified intent-to-treat [mITT] and per-protocol [PP] populations, respectively). The mixed-meal stimulation failed to distinguish between the groups. There was a trend toward efficacy in fasting C-peptide levels, though not statistically significant. Significantly more DiaPep277-treated than placebo-treated patients maintained target HbA_{1c} (mITT 56% versus 44%, *P* = 0.03; PP 60% versus 45%, *P* = 0.0082) and entered partial remission (mITT 38% versus 29%, *P* = 0.08; PP 42% versus 30%, *P* = 0.035). DiaPep277 treatment reduced the relative hypoglycemic event risk (mITT by 20%; PP by 28%).

CONCLUSIONS

DiaPep277 safely contributes to preservation of β-cell function and to improved glycemic control in patients with type 1 diabetes.

Diabetes Care 2014;37:1392–1400 | DOI: 10.2337/dc13-1391

[1]Diabetes Unit, Department of Internal Medicine, Hadassah University Hospital, Jerusalem, Israel
[2]Institute of Diabetes Research, Helmholtz Center, Klinikums Rechts der Isar, University of Technology, Munich, Germany
[3]University Hospital, Giessen, Germany
[4]Department of Medicine I, Rudolfstiftung Hospital, Vienna, Austria
[5]UCT Private Academic Hospital, Cape Town, South Africa
[6]Center of Diabetes Endocrinology, Johannesburg, South Africa
[7]Department of Endocrinology, University of Palermo, Palermo, Italy
[8]Department of Emergency and Organ Transplantation, Section of Internal Medicine, Endocrinology, Andrology, and Metabolic Diseases, University of Bari Aldo Moro, Bari, Italy
[9]Institute for Endocrinology and Diabetes, Schneider Children's Medical Center, Petach Tikvah, Israel
[10]Hospital Universitari Arnau de Vilanova, Lleida, Spain
[11]Internal Clinic, Faculty Hospital, Olomouc, Czech Republic
[12]Wolfson Medical Center, Holon, Israel
[13]Andromeda Biotech Ltd., Yavne, Israel
[14]Department of Immunology, Weizmann Institute of Science, Rehovot, Israel
[15]Department of Endocrinology and Diabetes, University Campus Bio-Medico, Rome, Italy
[16]Center of Diabetes, Barts and the London School of Medicine, Queen Mary University of London, London, United Kingdom

Corresponding author: Itamar Raz, ntv502@netvision.net.il.

Received 12 June 2013 and accepted 10 October 2013.

Clinical trial reg. no. NCT00615264, clinicaltrials.gov.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc13-1391/-/DC1.

A slide set summarizing this article is available online.

**A complete list of the members of the DIA-AID 1 Writing Group can be found in the Supplementary Data online.*

See accompanying articles, pp. 1173 and 1384.

Type 1 diabetes is caused by autoimmune destruction of pancreatic β-cells (1,2), resulting in severe decline in endogenous insulin secretion. Intensive insulin therapy reduces, but does not eliminate, the risks of complications from type 1 diabetes (3,4) and normal glycemic control is difficult to achieve long term (5). Thus considerable research effort is invested in the search for safe and effective means of downregulating the autoimmune destruction of β-cells to preserve residual function (6). Although some successful phase 2 studies of antigen specific and nonspecific immune therapeutic agents have been reported (7–13), there have been no reports of positive results in phase 3 studies (14–16). Just recently, two independent phase 2 trials failed to demonstrate the efficacy of interleukin-1β inhibition as a means to halt deterioration of β-cell function after the onset of type 1 diabetes (17). Consequently, no treatment modality for the preservation of β-cell function currently exists.

DiaPep277 is a 24 amino-acid peptide derived from human heat-shock protein 60 that has been demonstrated to modulate immunological attack on β-cells in the NOD mouse model of type 1 diabetes (18,19). The peptide induces anti-inflammatory T-cells and blocks destruction of β-cells while preserving insulin secretion (20,21), as was also demonstrated in a series of phase 2 studies (22–24). Additional studies demonstrated that DiaPep277 also activates regulatory T-cells by interacting with their Toll-like receptor 2 (25,26). Induction of regulatory T-cells diverts the immune response toward preservation of β-cells, rather than their destruction, without affecting general T-cell function in mice (27) and humans (22,28), thus enabling a specific treatment for type 1 diabetes without suppressing essential immunological functions.

The present DIA-AID 1 study was designed to evaluate the safety and efficacy of DiaPep277 in preserving endogenous production of insulin (as indicated by C-peptide secretion) in newly diagnosed type 1 diabetic adult patients who also receive insulin.

RESEARCH DESIGN AND METHODS

Study Design

The full trial protocol (protocol version 6, Supplementary Data) and consent documents were approved by independent ethics committees of the health authorities in each participating country. All patients provided written informed consent. DIA-AID 1 is a phase 3, multinational, balanced-randomized, double-blind, placebo-controlled, parallel-group study (Fig. 1 and Supplementary Fig. 1). Patients were males or females aged 16–45 years (inclusive) who had recently been diagnosed with type 1 diabetes (up to 4 months prior to recruitment). Major inclusion criteria were patients were on insulin treatment since diagnosis, had fasting C-peptide levels of ≥0.22 nmol/L, tested positive for the presence of at least one of the diabetes-related autoantibodies (IA-2 protein tyrosine phosphatase, glutamic acid decarboxylase, or insulin antibody), and had a BMI of 17–28 kg/m^2 (inclusive). Major exclusion criteria were pregnancy, significant diseases that could affect response to treatment, the presence of a known clinically significant immune deficiency, or treatment with immunosuppressive or cytotoxic drugs.

The sample size calculation for the primary efficacy end point was based on meta-analysis of all phase 2 clinical trials in newly diagnosed type 1 diabetic patients in which the glucagon stimulation test (GST) was the only method used for evaluating the C-peptide response (23,24). A sample size of 126 in each group would have 90% power to detect a significant difference between placebo and DiaPep277-treated groups. Assuming that up to 15% of patients might be lost to follow-up, in order to ensure an adequate safety database, the study was planned to randomize up to 500 patients (250 per treatment group).

Patients were screened (*N* = 679) and then randomized (*N* = 457) throughout the period of September 2005 to May 2009 in 46 outpatient centers in Europe (Austria, Czech Republic, Finland, France, Germany, Greece, Italy, Spain, and England), Israel, and the Republic of South Africa. Eligibility for enrolment in the study was determined during the screening phase (for parameters of patients screened for eligibility, see Supplementary Table 1). Study baseline values were obtained and the baseline mixed-meal tolerance test (MMTT) was conducted after randomization at the baseline visit when treatment was first administered (i.e., month 0). The baseline GST was conducted 1 month later, at month 1. Month 3 represented the baseline value for glycemic parameters data to allow time for newly diagnosed patients to be stabilized.

Study Procedures

A full description of the study procedures can be found in protocol version 6 (Supplementary Data). Briefly, patients were allocated randomly to receive drug or placebo subcutaneously at months 0, 1, 3, 6, 9, 12, 15, 18, and 21 of the study. Participants in the drug arm received DiaPep277 (1 mg) with mannitol (40 mg) in a 0.5 mL lipid emulsion, while patients in the placebo arm received only mannitol (40 mg) in a 0.5 mL lipid emulsion. The total study duration was 25 months, including screening, treatment, and follow-up (see Supplementary Fig. 1).

Participant randomization was performed by an independent third party, Almac Clinical Technologies (Craigavon, U.K.), which randomly assigned each patient a number linked to one of the treatment arms and to a medication kit. This company also managed the assignment of participants at all sites via a controlled interactive voice/Web response system to maintain a treatment randomization ratio of 1:1 (DiaPep277:placebo) at each study site and across the trial as a whole. Randomization was stratified by basal fasting C-peptide concentrations (C-peptide ≥0.22 nmol/L or C-peptide ≥0.40 nmol/L) and HbA_{1c} (≥7% [≥53 mmol/mol] or <7% [<53 mmol/mol]), resulting in a total of four independently randomized pretreatment strata.

Participants, investigator site staff, persons performing the assessments, and data analysts were blinded to patient allocation from the time of randomization until database lock.

Outcome Measures

Safety and tolerability assessments were performed on the intent-to-treat (ITT) population. These included

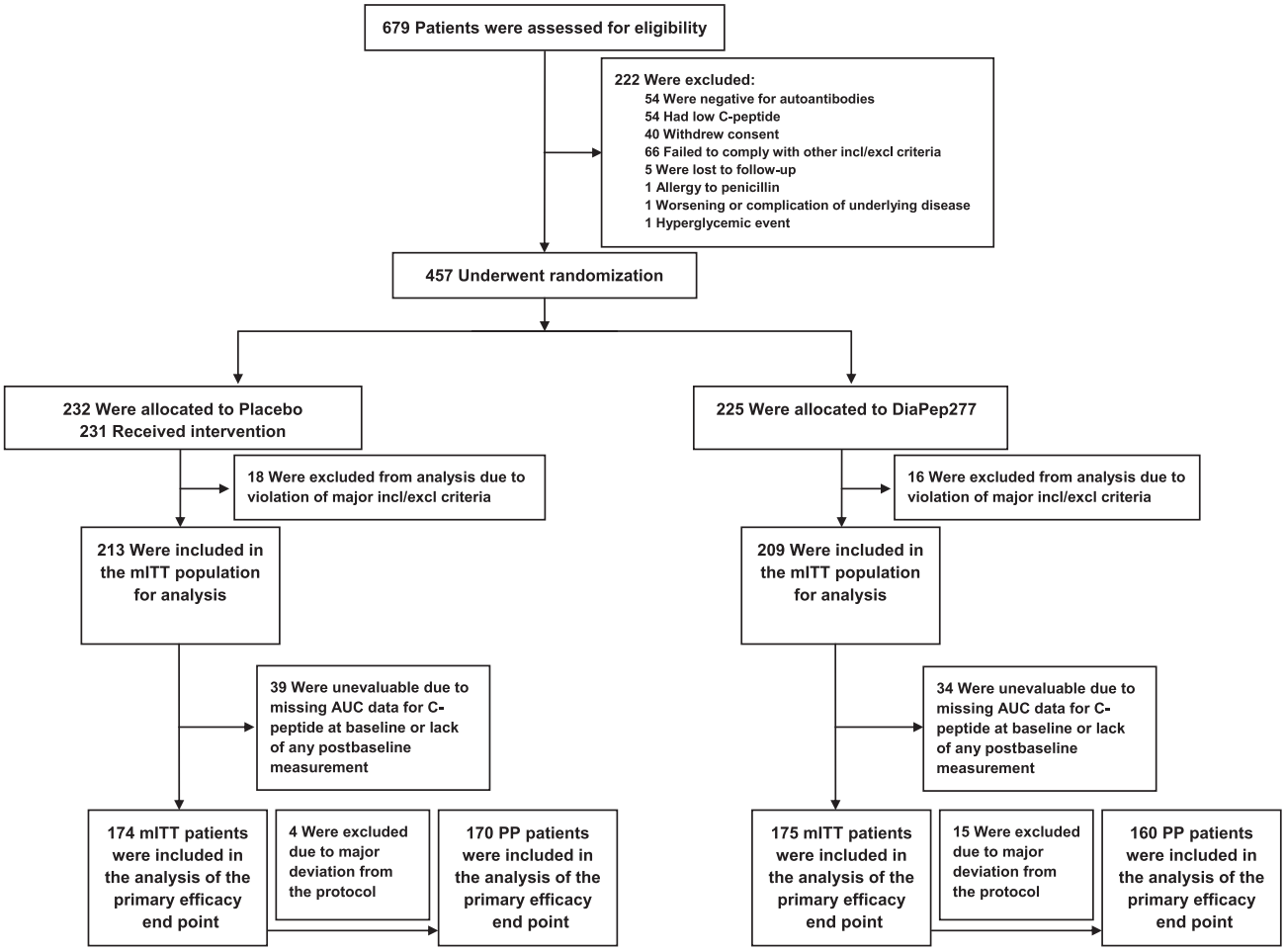

Figure 1—Flow diagram of the study. Patients recruited at 46 outpatient centers in 11 countries were screened (679), randomized (457), and assigned to either the placebo or treatment. The mITT consisted of 422 patients after excluding 34 patients who were erroneously randomized in violation of major inclusion/exclusion criteria. Missing data resulted in a further 73 patients being excluded from analysis for the primary end point. Severe protocol violations occurred in 19 patients, who were excluded in the PP analysis. Incl/excl, inclusion/exclusion.

assessment of adverse events (AEs), serious AEs and treatment-emergent adverse events (TEAEs), as classified by the *Medical Dictionary for Regulatory Activities*; clinical laboratory test results; vital sign measurements; electrocardiography; physical examination; dermal hypersensitivity; and hypoglycemic event rate.

Efficacy analyses were performed on the modified ITT (mITT) and per-protocol (PP) populations. C-peptide was used as a marker of insulin secretion and thus of residual β-cell function (29). For the primary efficacy end point, patients were evaluated if they had the area under the glucagon-stimulated C-peptide curve ($AUC_{0–20}$) for baseline and at least one efficacy end point evaluation post baseline. The prespecified study populations were defined as follows: ITT, all randomized patients who received at least one dose of study medication; mITT, all ITT patients who entered the study without violation of major inclusion and exclusion criteria; and PP, all mITT patients who participated in the study without significant deviation in compliance from the study protocol.

At the time of study initiation (2005) there was no published guidance regarding the preferred method for C-peptide stimulation. The evaluation of DiaPep277 efficacy in all the phase 2 studies was based on GST, which is a valid and commonly used procedure. Consequently, the sample size calculation for the phase 3 study was based on the variance of the GST procedure. Despite this, the MMTT was selected as the primary method of C-peptide stimulation because of its growing popularity with experts and the supposition that eventually it will become the preferred method in intervention studies.

While the DIA-AID 1 study was still blinded and ongoing, the results of a phase 2 study in DiaPep277-treated latent autoimmune diabetes of adult onset (LADA) patients that used both the GST and MMTT methods of stimulation were reported. To our surprise, the results of this study showed clear and unexpected differences between the changes in C-peptide when measured by MMTT versus GST [described in detail by Pozzilli et al. (30)].

These differences and the fact that the sample size of the DIA-AID 1 study was calculated based on the glucagon stimulation results from the phase 2

studies prompted our Clinical Advisory Board to recommend changing the method of measurement of the primary end point.

Accordingly, the change from baseline in the area under the 20-min GST-stimulated C-peptide secretion curve ($AUC_{0–20}$) was redefined as the primary efficacy outcome measure. The change from baseline in the area under the 120-min MMTT-stimulated curve ($AUC_{0–120}$) was retained as a secondary outcome measure.

The study protocol was amended, and the statistical analysis plan was planned and finalized before the study was unblinded, with the GST clearly defined as the primary end point.

This is the first large trial in which long-term changes in stimulated C-peptide secretion levels were followed using both measurement methods.

Additional secondary measures were the percentage of patients maintaining a treat-to-target HbA_{1c} level of ≤7% (≤53 mmol/mol) at study end and the difference in β-cell function between the DiaPep277 and the placebo groups, as determined by the change in fasting C-peptide levels from baseline to 24 months. Exploratory clinical end points included the proportion of patients who achieved partial remission, defined as HbA_{1c} ≤7% (≤53 mmol/mol), with insulin doses of ≤0.5 units/kg/day at study end, and the number and rate of hypoglycemic events during the study.

All laboratory tests were performed in central laboratories. All samples, including urine, hematology, blood chemistry, C-peptide, and HbA_{1c}, were processed at LKF GmbH, Schwentinental, Germany.

Two tests were used to measure C-peptide secretion: the intravenous GST and the 2-h MMTT. β-Cell function was assessed by measurements of basal fasting C-peptide at each visit, intravenous glucagon-stimulated C-peptide secretion at baseline (month 1) and months 12 and 24, and MMTT-stimulated C-peptide secretion at baseline (month 0) and months 6, 12, 18, and 24. Basal fasting and stimulated C-peptide measurements were performed in the morning after an overnight fasting period of 8 to 10 h. The tests were performed only if the fasting glucose was in the range of 4 to 11.1 mmol/L (72 to 200 mg/dL). Investigators were blinded to the GST and MMTT results in order to maintain the blind to study treatment.

Glucagon (GlucaGen HypoKit, Novo Nordisk), 1 mg, was administered intravenously at time "0" within 15 s. Blood samples for the determination of C-peptide were drawn at –5 min before and immediately before glucagon administration (time 0) and at 2, 6, 10, and 20 min postadministration (±5 min).

The standard MMTT consisted of the oral ingestion of a standardized liquid mixed meal (Ensure, Abbott; calculated for each patient as 6 mL/kg body weight, up to 360 mL). Blood samples for the determination of C-peptide were drawn at −10 min before and immediately before the liquid meal ingestion (time 0) and at 10, 20, 30, 60, 90, and 120 min (±5 min) postingestion.

C-Peptide was measured using radioimmunoassay kit (Human C-peptide RIA kit, LINCO). The analytical range of the assay is 0.0331–1.655 nmol/L.

Autoantibodies associated with type 1 diabetes were tested at the Diabetes Research Center of the Free University of Brussels, Belgium, under the supervision of Dr. Patrick Goubert and Prof. Frans Gorus.

Data Analysis

All statistical methods complied with *Guidance for Industry: E9 Statistical Principles for Clinical Trials* (31). Analyses were performed using SAS version 9.2 or above for Windows. Outcomes are reported for two populations: an mITT group and a PP group. The mITT group was created [in line with Fergusson et al. (32)] after data lock and before study unblinding to correct for the mistaken randomization of 34 ineligible patients into the trial (Fig. 1 and Supplementary Table 2). The PP population consisted of all mITT patients who did not significantly deviate from the study protocol.

The mixed-effects model for repeated measurements (MMRM) was used as the first statistical method for analysis of efficacy and was applied to all the patients in both the mITT and the PP groups. Every participant for whom there existed at least one postbaseline measurement was included in the MMRM analysis, with the model imputing the missing data points (33,34). The MMRM was used to analyze the change from baseline in the primary and secondary end points using SAS PROC MIXED. The model included fixed effects for baseline fasting C-peptide and categorical model terms for treatment group, visit number, and country. A variance components matrix was used to model the covariance structure. *P* values were obtained from differences of least square means at each visit. A *P* value of <0.05 was considered significant.

Relative treatment effect was defined as the ratio between the changes in area under the curve (AUC) in the DiaPep277-treated group compared with the placebo group. The hypoglycemic events data were analyzed using paired *t* tests to compare the value for each patient at baseline (month 3) with the value at 24 months. For detailed calculations, see Supplementary Data. For hypoglycemia classifications see Supplementary Table 3.

Interim Analysis

An interim analysis intended for sample size re-estimation was performed when approximately 100 patients reached 24 months. No stopping rules were set up for early termination and no type I error adjustment was carried out. All the type I errors will be spent in the final analysis. An interim analysis data safety monitoring board (IA-DSMB) was appointed to review the results of the interim analysis. The unblinded outputs of the interim analysis were not accessed by any person other than the members of the IA-DSMB.

The IA-DSMB conclusions and recommendations following the interim analysis were to continue the study as planned.

RESULTS

Two hundred twenty-two patients screened were not eligible for the trial, mainly because they lacked autoantibodies (24.3%) or had low

C-peptide levels (24.3%) (Fig. 1 and Supplementary Table 4). The treatment and placebo arms were comparable in terms of demographic data, medical history data, and baseline metabolic and β-cell function parameters (Table 1). The higher male preponderance (66% males, 34% females) was mainly because female participants were required to use a birth control method throughout the 2-year study duration.

Three hundred forty-nine patients from the mITT population and 330 patients from the PP population were evaluable for the primary efficacy end point (i.e., data were available at baseline and at least one time point postbaseline) (Fig. 1).

One hundred two patients (22%) did not complete the study, 50 from the DiaPep277-treated arm and 52 from the placebo-treated arm. The main reasons were withdrawal of consent (7.8%), loss to follow-up (4%), noncompliance with study requirements (2%), and AE (2%). Withdrawal for any reason was balanced between the two treatment arms (see Supplementary Table 5). Noncompleters who had at least one time point postbaseline were evaluable for the primary efficacy end point.

Efficacy Analysis

DiaPep277-treated patients maintained significantly higher GST-stimulated C-peptide secretion levels than did placebo-treated patients at the end of the study, month 24. Significantly less decline in $AUC_{0–20}$ from baseline to study end was observed in mITT patients who were treated with DiaPep277 as compared with placebo-treated patients (−3.108 vs. −4.058; difference 0.949 [95% CI 0.056–1.843] nmol/L/20 min; $P = 0.037$), which equates to a relative treatment effect of 23.4% (Fig. 2*A*). A similar result was obtained in the PP population (−2.857 in the DiaPep277-treated group vs. −4.037 in the placebo-treated group; difference 1.180 [95% CI 0.271–2.088] nmol/L/20 min; $P = 0.0011$), a relative treatment effect of 29.2% (Fig. 2*B*).

Unlike the results obtained in the GST-stimulated C-peptide secretion, the MMTT-stimulated C-peptide secretion did not differ between the groups (Fig. 2*C*).

From the GST-stimulated C-peptide concentration curve, the maximum C-peptide concentration (C_{max}) was extracted. The change from baseline in C_{max} was consistent with the change in C-peptide $AUC_{0–20}$. In both the mITT and PP populations, the DiaPep277-treated group exhibited less decline in C_{max} than the placebo-treated group (−0.185 vs. −0.247, difference 0.062 [95% CI 0.005–0.118] nmol/L/20 min, $P = 0.032$ for a relative treatment effect of 25% in the mITT population and −0.166 vs. −0.246, difference 0.08 [95% CI 0.024–0.137] nmol/L/20 min, $P = 0.005$ for a relative treatment effect of 32% in the PP population) (Supplementary Fig. 2).

A relative treatment effect of 20% was obtained for the difference in fasting C-peptide levels between the treatment and placebo groups at study end. Although not statistically significant, it supports the treatment effect obtained by GST.

A significantly higher proportion of patients maintained HbA_{1c} ≤7% (≤53 mmol/mol) at study end in the DiaPep277-treated group as compared with the placebo group in both the mITT population (56 and 44%, respectively; $P = 0.03$) (Fig. 3*A*) and the PP population (60 and 45%, respectively; $P = 0.0082$) (Fig. 3*B*). As a result of the tight glycemic control of patients that was required throughout the study and maintained by administering maximal insulin to prevent β-cell glucotoxicity, the mean HbA_{1c} levels of the groups at study end were not expected to be significantly different: −7.32% (56 mmol/mol) and 7.11% (54 mmol/mol) in the DiaPep277-treated group compared with 7.39% (57 mmol/mol) and 7.30% (56 mmol/mol) in the placebo group for the mITT and the PP populations, respectively.

An exploratory analysis revealed that, in both the mITT and PP populations, there was a higher proportion of patients in partial remission at study end in the DiaPep277-treated group than in the placebo group. Specifically, in the mITT population, 38.4% of patients achieved partial remission compared with 29.3% in the placebo population ($P = 0.08$). The corresponding values for the PP

Table 1—Demographic and baseline parameters of randomized patients

Parameter	DiaPep277	Placebo
Sex (male/female)	142/83	158/73
Age (years)	26.6 ± 7.99 (15–46)	26.4 ± 7.85 (15–46)
Time since diagnosis (months)	2.76 ± 1.2 (0.77–7.6)	2.8 ± 1.3 (0.5–7.4)
BMI (kg/m^2)	22.87 ± 3.0 (17–35.4)	23.02 ± 3.1 (17–34.4)
Fasting C-peptide (nmol/L)	0.38 ± 0.22 (0.03–1.62)	0.39 ± 0.28 (0.05–3.4)
Baseline $AUC_{0–20}$ by GST (nmol/L/20 min)	10.81 ± 7.21 (1.39–78.01)	10.61 ± 6.48 (1.05–49.53)
Baseline $AUC_{0–120}$ by MMTT (nmol/L/120 min)	106.49 ± 60.28 (19.8–620.3)	100.18 ± 53.15 (12.25–398.5)
HbA_{1c} (%)	7.27 ± 1.65 (4–16.4)	7.50 ± 1.70 (4.5–14.6)
HbA_{1c} (mmol/mol)	56.0 ± 18.0 (20–156)	58.4 ± 18.9 (26–136)
Insulin (units/kg/day at 3 months)	0.42 ± 0.30 (0.03–2.02)	0.43 ± 0.27 (0.02–2.0)
Autoantibodies (% positive)		
IA-2A	57.8%	62%
IA	74.7%	73.3%
GADA	88.4%	83.2%

Data are average ± SD (range) unless otherwise specified. IA-2A, IA-2 protein tyrosine phosphatase; IA, insulin antibody; GADA, glutamic acid decarboxylase.

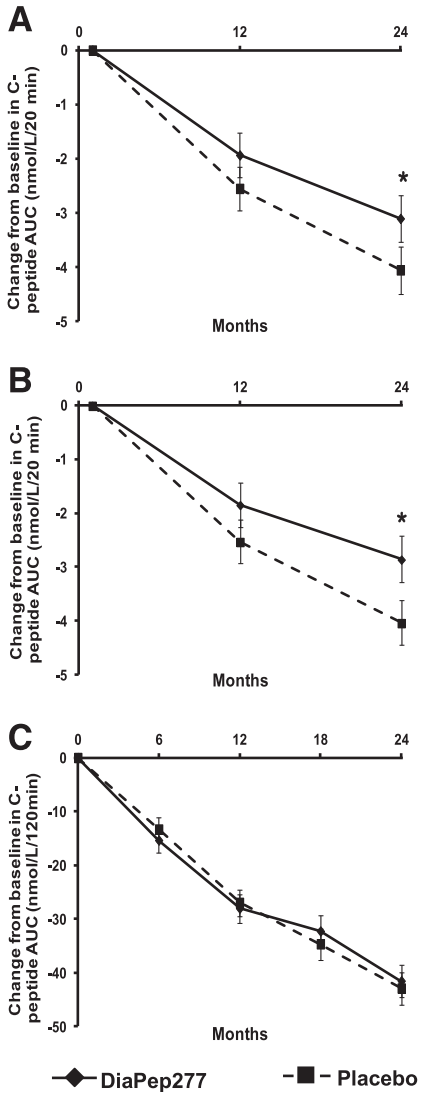

Figure 2—Change from baseline (±SEM) in the area under the C-peptide secretion curve as determined using the 20-min GST ($AUC_{0–20}$) in (*A*) the mITT population and (*B*) the PP population. (*C*) Change from baseline (±SEM) in the area under the C-peptide secretion curve as determined using the 120-min mixed-meal stimulation test ($AUC_{0–120}$) in the mITT population. *$P < 0.05$.

population were 41.8 and 30.2% (P = 0.035) for the treatment and placebo groups, respectively (see Supplementary Table 6).

There were fewer hypoglycemic events per month in the mITT population in the DiaPep277-treated group compared with the placebo group (69 vs. 83, respectively) as well as fewer hypoglycemic events per month per patient (0.57 vs. 0.71, respectively). The same trends were observed in the PP population (55 vs. 83 hypoglycemic events per month and 0.51 vs. 0.71 hypoglycemic events per month per patient for the treatment and placebo groups, respectively). The decrease in the rate of hypoglycemic events from month 3 to study end in the DiaPep277-treated group was significant (−0.18, P = 0.012 for the mITT population and −0.26, P = 0.0004 for the PP population), while there was no significant change in the placebo-treated group (−0.04, P = 0.644 for both the mITT and PP populations) (Fig. 3*C* and *D*). Indeed, treatment with DiaPep277 reduced the overall relative risk of a patient suffering a hypoglycemic episode over the 2 years of the study by 20% in the mITT population and by 28% in the PP population.

Safety

The most common AE considered related to the study drug was discomfort at the injection site upon administration. No laboratory abnormalities, changes in vital signs, general immune system suppression, increases in infections or autoimmune diseases, or significant differences in serious AE or AE frequencies were observed between treated and untreated patients. In the DiaPep277-treated group, three patients (1.3%) experienced at least one serious TEAE that was considered to be related to the study drug, namely, diverticulitis, erythema nodosum, and hypoglycemia. In the placebo group, one patient (0.4%) experienced at least one serious TEAE that was considered to be related to the study drug, namely, neutropenia. Twenty-three patients (10.2%) in the DiaPep277-treated group and 13 patients (5.6%) in the placebo group experienced at least one serious TEAE not considered to be drug related. All TEAEs resolved completely (see Supplementary Table 7).

There were three cases of serious hypoglycemia in two subjects in each the DiaPep277-treated and the placebo-treated group. In the case of the DiaPep277-treated subject who suffered two incidences of serious hypoglycemia, the investigator reported them as drug related. There was one case of hypoglycemic coma in each the DiaPep277-treated and the placebo-treated group considered not to be drug related.

CONCLUSIONS

The current study shows that DiaPep277 has an excellent safety profile, in line with previous studies (22–24). Over 500 type 1 diabetic patients have been exposed to DiaPep277 for up to 2 years without safety concerns. Subjects treated with DiaPep277 were not immunocompromised and were spared the side effects characteristic of immunosuppressive drugs, which suggests that DiaPep277 treatment at 3-monthly intervals could be continued as long as required. A recently completed extension study showed that up to 4 years of continued treatment has not led to any drug-related health concerns (35). Additional studies will determine whether and to what extent continued treatment is needed to maintain long-term treatment effect.

A most encouraging result of this study is the improved clinical outcome related to better glycemic control, as shown by a significant increase in the proportion of patients maintaining the target HbA_{1c} level at study end in the DiaPep277-treated group. It is important to note that this was attained without any increase in insulin dose, as evidenced by the higher number of patients in the DiaPep277-treated group who achieved partial remission. Moreover, the number of hypoglycemic events per month in the DiaPep277-treated group decreased over the course of the study, in contrast to the placebo-treated group, whose rate remained unchanged. This improved metabolic control in the DiaPep277-treated group most likely resulted from the preservation of a clinically significant degree of endogenous insulin secretion, as evidenced by a smaller decline in C-peptide $AUC_{0–20}$ secretion in response to glucagon stimulation.

Initially, the MMTT was selected as the primary method for measuring efficacy and the GST as a secondary method. During the course of the study, while it was still blinded, it became apparent that there might be discrepancies between the two methods (as explained in detail in OUTCOME MEASURES). These differences and the fact that the sample size of the DIA-AID 1 study was calculated based on the glucagon stimulation results from the phase 2

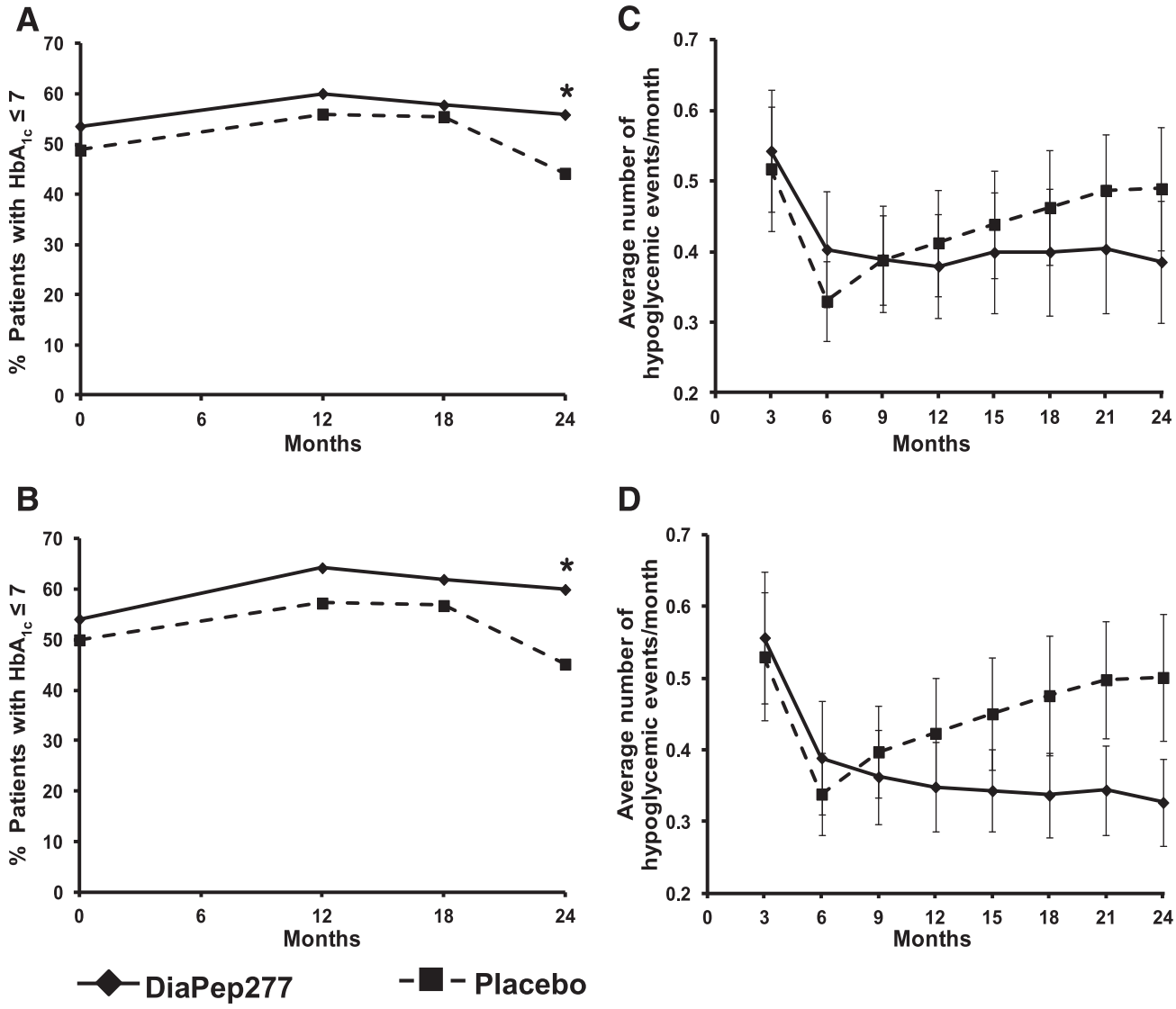

Figure 3—The percentage of patients achieving HbA_{1c} ≤7% (53 mmol/mol) in (*A*) the mITT population and (*B*) the PP population. The average number (±SEM) of hypoglycemic events per month in (*C*) the mITT population and (*D*) the PP population. The number of hypoglycemic events per month at baseline was computed as the total number of events recorded up until month 3 divided by the number of months (3). Thereafter, this value was computed as the total number of hypoglycemic events from month 3 until the date of the specified visit divided by the number of months, on the basis of 30-day months. *$P < 0.05$.

studies prompted us to redefine the primary end point as the change in C-peptide AUC from baseline to study end after stimulation with glucagon. The change in MMTT-stimulated C-peptide was redefined as a secondary end point.

While GST-stimulation showed that DiaPep277 treatment yielded, in addition to positive clinical outcomes, preservation of β-cell function, the MMTT-stimulation failed to detect a difference between the two study groups.

Correlation analyses indicated that although the absolute values of stimulated C-peptide measured by GST and MMTT were reproducible and well correlated when evaluated at each individual time point, the changes in AUC obtained by the two methods over the course of the study were only weakly correlated. Since the change in AUC over time from baseline (rather than absolute AUCs) indicate the dynamics of disease progression, only this parameter should be used to calculate treatment effect and preservation of residual β-cell function in intervention studies.

Additional analyses indicated that the differences between the outcome measures are statistically robust and may reflect differences in the biological response to the stimulations.

This inconsistency between the GST- and MMTT-stimulated C-peptide results has not been reported in other long-term intervention trials, because no other trials have evaluated patients by both procedures. This is the first long-term intervention trial that uses both procedures simultaneously and so enables such an observation.

The discrepancy between the GST and the MMTT results is described in detail by Pozzilli et al. (30).

A second, confirmatory phase 3 study is ongoing using both methods of stimulation. This will allow the collection of a large amount of data that could possibly aid in understanding the discrepancy between the two methods. The ability of DiaPep277 to arrest the autoimmune destruction of β-cells at the early nonsymptomatic state should also be explored.

Acknowledgments. The heat-shock protein–derived peptide, p277, was developed and its role in arresting β-cell destruction in type 1 diabetes was identified in the laboratory of I.R.C. at the Weizmann Institute of Science, Israel.

Duality of Interest. This clinical study was funded by Andromeda Biotech. I.R. is a consultant to Andromeda Biotech and was head of the steering committee for the trial. T.L. is a consultant to Andromeda Biotech. F.B. is a consultant to Andromeda Biotech. D.E., A.A., M.T., R.E., D.P., and S.D. are employees of Andromeda Biotech. I.R.C. is a consultant to Andromeda Biotech. D.E. and I.R.C. are also the inventors of DiaPep277. P.P. is a consultant to Andromeda Biotech and received travel grants. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. I.R. wrote the manuscript and contributed to study implementation and supervision of data collection at the sites. A.G.Z., F.B., L.A.D., C.G., F.G., L.d.V., D.M., V.P., and J.W. contributed to study implementation and supervision of data collection at the sites. T.L., G.S., and P.P. contributed to the discussion, reviewed and edited the manuscript, and contributed to study implementation and supervision of data collection at the sites. D.E., A.A., R.E., and S.D. researched data, contributed to the discussion, and reviewed and edited the manuscript. M.T., D.P., and I.R.C. contributed to the discussion and reviewed and edited the manuscript. All authors had full access to the data, contributed to data interpretation, and reviewed and approved the final version of this article. Andromeda Biotech and i3 Research (a contract research organization) conducted and managed the study. Data were analyzed by the Biostatistics Department at i3 Research. I.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in abstract form at the following scientific meetings: the 7th International Congress of Autoimmunity, Ljubljana, Slovenia, 5–9 May 2010; the 46th Annual Meeting of the European Association for the Study of Diabetes, Stockholm, Sweden, 20–24 September 2010; the World Diabetes Congress, Dubai, United Arab Emirates, 4–8 December 2011; the 8th International Congress of Autoimmunity, Granada, Spain, 9–13 May 2012; the 72nd Scientific Sessions of the American Diabetes Association, Philadelphia, PA, 8–12 June 2012; and the 12th International Congress of the Immunology of Diabetes Society, City of Victoria on Vancouver Island, Canada, 15–19 June 2012.

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