ORIGINAL ARTICLE

GAD65 Antigen Therapy in Recently Diagnosed Type 1 Diabetes Mellitus

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ABSTRACT

BACKGROUND

The 65-kD isoform of glutamic acid decarboxylase (GAD65) is a major autoantigen in type 1 diabetes. We hypothesized that alum-formulated GAD65 (GAD-alum) can preserve beta-cell function in patients with recent-onset type 1 diabetes.

METHODS

We studied 334 patients, 10 to 20 years of age, with type 1 diabetes, fasting C-peptide levels of more than 0.3 ng per milliliter (0.1 nmol per liter), and detectable serum GAD65 autoantibodies. Within 3 months after diagnosis, patients were randomly assigned to receive one of three study treatments: four doses of GAD-alum, two doses of GAD-alum followed by two doses of placebo, or four doses of placebo. The primary outcome was the change in the stimulated serum C-peptide level (after a mixed-meal tolerance test) between the baseline visit and the 15-month visit. Secondary outcomes included the glycated hemoglobin level, mean daily insulin dose, rate of hypoglycemia, and fasting and maximum stimulated C-peptide levels.

RESULTS

The stimulated C-peptide level declined to a similar degree in all study groups, and the primary outcome at 15 months did not differ significantly between the combined active-drug groups and the placebo group ($P=0.10$). The use of GAD-alum as compared with placebo did not affect the insulin dose, glycated hemoglobin level, or hypoglycemia rate. Adverse events were infrequent and mild in the three groups, with no significant differences.

CONCLUSIONS

Treatment with GAD-alum did not significantly reduce the loss of stimulated C peptide or improve clinical outcomes over a 15-month period. (Funded by Diamyd Medical and the Swedish Child Diabetes Foundation; ClinicalTrials.gov number, NCT00723411.)

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The clinical onset of type 1 diabetes is manifested by the effects of inadequate insulin secretion due to the immunologic destruction of pancreatic-islet beta cells.[1] Despite replacement therapy with exogenous insulin, type 1 diabetes is associated with substantial morbidity and mortality.[2,3] Even modest preservation of insulin secretion appears to reduce short- and long-term complications of type 1 diabetes.[4-9] Initial attempts at immunosuppression to treat type 1 diabetes had a positive effect but one that was outweighed by treatment-related adverse events.[10-12] More recently, selective immunosuppression has been attempted. Phase 2 trials showed promising efficacy, but phase 3 studies have been less encouraging.[13-17]

Autoantigens have been proposed as an alternative approach to the treatment of type 1 diabetes, to induce immunologic tolerance.[18] Parenteral insulin as well as nasal insulin had no effect when used to prevent the disease.[19,20] In the Diabetes Prevention Trial–Type 1, treatment with oral insulin did not result in achievement of the primary end point (prevention of type 1 diabetes), although it may have prevented the disease in persons with high concentrations of insulin autoantibodies.[21] The heat-shock peptide DiaPep 277 has been reported to have some positive effects in adults, but not children, with newly diagnosed type 1 diabetes.[22,23]

In our previous study, treatment with the 65-kD isoform of glutamic acid decarboxylase (GAD65) formulated with alum (GAD-alum) was associated with a preserved fasting C-peptide level at 30 months after treatment and a preserved stimulated C-peptide level at 15 months.[24] The greatest and most persistent efficacy was in patients treated for less than 6 months after diagnosis; these patients still had preservation of the fasting C-peptide level after 4 years.[25] More recently, a phase 2 trial of GAD-alum in patients with newly diagnosed type 1 diabetes did not show any clinical benefit.[26] We conducted a phase 3 trial of GAD-alum treatment initiated within 3 months after the diagnosis.

Methods

Study Conduct

The study was designed by the academic authors with representatives of the sponsor, Diamyd Medical. Data were gathered from clinical investigators by the monitors. Diamyd Medical provided the GAD-alum and matching placebo and was also involved in the conduct and management of the trial, including data collection and analysis, both directly and through multiple contract research organizations. The first author vouches for the data, the analysis, and the fidelity of the report to the study protocol.

The study was approved by the relevant regulatory authorities and research ethics boards for the participating sites and countries. All patients provided written informed consent or assent (the latter in combination with consent provided by a parent or guardian). The study was conducted in accordance with the protocol and the statistical-analysis plan, both of which are available with the full text of this article at NEJM.org.

Patient Recruitment

This study was a multicenter, randomized, double-blind trial performed at 63 clinics in nine European countries (Finland, France, Germany, Italy, the Netherlands, Slovenia, Spain, Sweden, and the United Kingdom). The recruitment ratio was 1:1:1 across the three study groups, with an intended total enrollment of 320 patients. All patients were monitored for safety, and an independent data and safety monitoring board met at least every 6 months.

Patients with recent-onset type 1 diabetes who were 10 to 20 years of age were screened between August 2008 and November 2009. Inclusion in the trial required detectable serum GAD65 autoantibodies, a fasting C-peptide level above 0.3 ng per milliliter (0.1 nmol per liter), and a duration of type 1 diabetes of less than 3 months.

Study Outcomes

The primary outcome was the change in the stimulated serum C-peptide level (mean area under the curve [AUC] over the 2-hour period after a mixed-meal tolerance test) between the baseline visit and the 15-month visit.[27] Secondary outcomes included the changes in the mean daily insulin dose, glycated hemoglobin level, and fasting C-peptide level; the incidences of any hypoglycemic event and of severe hypoglycemic events (i.e., hypoglycemia with unconsciousness, convulsions, or both); changes in the stimulated C-peptide level between baseline and 30, 60, 90, and 120 minutes during the mixed-meal tolerance test; the proportions of patients with a maximum stimulated C-peptide level greater than 0.6 ng per milliliter (0.2 nmol

per liter); and the proportion of patients with a glycated hemoglobin value below 7%. The secondary outcomes of insulin-dose–adjusted glycated hemoglobin and incidence of severe hypoglycemic events were added after commencement of the trial.

Preplanned exploratory subgroup analyses included stratification according to the baseline characteristics of sex, age, body-mass index, maximum stimulated C-peptide level, glycated hemoglobin level, days since diagnosis of type 1 diabetes, risk related to HLA type, country, region within Europe, pubertal stage, GAD65 autoantibody level, fasting C-peptide level, and insulin dose.

STUDY TREATMENTS AND PROCEDURES

Three regimens were administered: subcutaneous injections of 20 μg of GAD-alum on days 1, 30, 90, and 270 (four-dose regimen); subcutaneous injections of GAD-alum on days 1 and 30 and of placebo on days 90 and 270 (two-dose regimen); and injections of placebo on days 1, 30, 90, and 270.

Randomization to one of the three regimens was stratified by country and performed in balanced blocks of six. A computer-generated randomization list was produced by Perceptive Informatics (www.perceptive.com/clinphone-rtsm). For each visit when study medication was to be administered, the study investigator called Perceptive Informatics, which assigned a vial number to the patient for use during that visit. The placebo and active drug product were both suspensions of alum in a buffer in identical vials. Each vial was packed in a vial box, with the box and the vial labeled with the same vial number. Patients, investigators, and study personnel remained unaware of the study-regimen assignments during the 15 month primary efficacy period.

Medical assessments were performed on day 1 and at months 1, 3, 9 and 15 of the study. Mixed-meal tolerance tests were performed on day 1 and at months 3, 9, and 15. Neurologic assessments were performed at the screening visit and at months 3, 9, and 15. For all visits after the screening visit, GAD65 autoantibody levels and insulin-dose information for the 4 days before each visit were collected. Investigator and patient assessments of injection-site reactions were collected before and after each injection. Patients documented all possible hypoglycemic events in diaries, which were collected at each study visit. HLA class II typing was performed at the 1-month visit.

LABORATORY TESTS

All serum, urine, GAD65 autoantibody, and C-peptide analyses were performed centrally by BARC Laboratories (Ghent, Belgium). GAD65 autoantibody levels were assessed by means of enzyme-linked immunosorbent assay (GAD65 Antibody ELISA, RSR), with results measured on a microtiter plate reader (PowerWave HT, Biotek). C-peptide quantification was performed (with the use of an Immulite 2000 C-peptide kit and analyzer) with calibration standards based on the World Health Organization's National Institute for Biological Standards International Reference Reagent standards (product number, 84/510). HLA typing was performed (with the Inno Lipa Kit, Innogenetics) after extraction of DNA (by means of EasyMag, Biomérieux), and polymerase-chain-reaction assays were performed (on an Autolipa 48 instrument) for HLA detection.

STATISTICAL ANALYSIS

We estimated that 93 patients in each of the three study groups would provide 90% power to detect a 45% difference in the primary end point between each of the two GAD-alum groups and the placebo group at a two-sided significance level of 5%. This estimate is based on a t-test of log-transformed data with a standard deviation of 0.718, obtained from the value in a phase 2 study of GAD-alum in patients with newly diagnosed type 1 diabetes and by applying Dunnett's adjustment for two comparisons.[24] Taking into account the possibility that 12.5% of patients would not have a postbaseline assessment, we planned to randomly assign a total of 320 patients to the three groups in equal numbers. No interim analyses for efficacy or futility were planned or performed.

The statistical analysis was based on the predefined modified intention-to-treat population: all randomized patients who received at least one dose of study medication and had at least the baseline and one postbaseline assessment of primary efficacy variables. The statistical method used to test each of the hypotheses was a restricted maximum-likelihood–based repeated-measures approach (mixed-model repeated measures).[28,29] The model for analysis included fixed, categorical effects of study drug, country, visit, and interaction of study drug by visit, as well as the continuous, fixed covariates of baseline value and interaction of baseline value by visit. Patient identification number was included

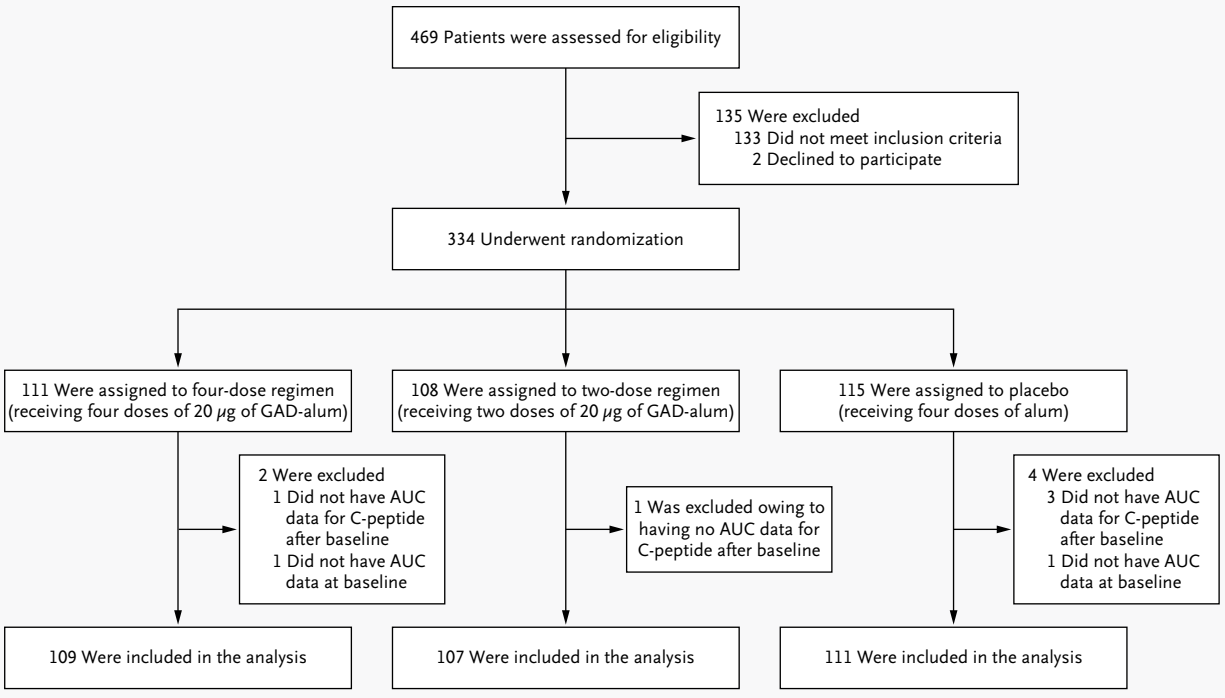

Figure 1. Enrollment, Randomization, and Follow-up of the Figure 2. C-Peptide and GAD65 Autoantibody Levels, According to Study Group.
Mean changes in stimulated C-peptide levels are shown in Panel A, and median GAD65 autoantibody levels are shown in Panel B. In Panel A, the I bars indicate standard deviations. In Panel B, the two-sided P value is shown for the four-dose regimen and the two-dose regimen combined as compared with placebo. To convert values for C-peptide to nanograms per milliliter, divide by 0.333.

as a random factor. A covariance–variance structure was used to model the within-patient errors. The covariance–variance structure converging to the best fit, as determined by Akaike's information criterion, was used. The Kenward–Roger approximation was used to estimate denominator degrees of freedom. Missing data were modeled on the patient's available data and on other patients' developments over time. The primary study comparisons, between each active treatment and placebo at month 15, were based on least-squares means. Nominal P values are reported, along with the two-sided 95% confidence intervals, for the estimated between-group differences.

RESULTS

ENROLLMENT AND RANDOMIZATION

Of the 469 patients assessed for eligibility, 334 underwent randomization (Fig. 1). The modified intention-to-treat population comprised 327 patients: 109 who received four doses of GAD-alum (the four-dose regimen), 107 who received two doses of GAD-alum (the two-dose regimen), and 111 who received placebo. Of the 133 patients who were screened but not enrolled, 67 did not meet the eligibility criterion for residual fasting C-peptide level, and 74 did not meet the eligibility criterion for elevated GAD65 autoantibody level. Seven study patients were not included in the analysis (2 in the four-dose group, 1 in the two-dose group, and 4 in the placebo group) because of missing data on the stimulated C-peptide level at baseline or at 15 months.

BASELINE CHARACTERISTICS

The baseline characteristics of the patients (Table 1) were generally well balanced among the three study groups. However, randomization was not stratified for age, and the distribution of age groups varied among the three study groups. The percentages of patients who were 10 to 11 years of age were 33.9%, 31.8%, and 23.4% in the four-dose group, the two-dose group, and the placebo group, respectively, whereas the corresponding percentages for patients 16 to 20 years of age were

Table 1. Baseline Characteristics of the Patients, According to Study Group.*

Characteristic	4-Dose Regimen (N=111)	2-Dose Regimen (N=108)	Placebo (N=115)
Age — yr	12.9±2.4	12.9±2.1	13.3±2.3
Time from diagnosis to first treatment — days	73.6±25.0	76.9±23.3	74.2±23.4
Sex — no. (%)			
Female	58 (52.3)	48 (44.4)	55 (47.8)
Male	53 (47.7)	60 (55.6)	60 (52.2)
BMI percentile — no. (%)	111	107	114
<10.0	9 (8.1)	14 (13.1)	7 (6.1)
10.0–24.9	11 (9.9)	7 (6.5)	13 (11.4)
25.0–49.9	27 (24.3)	25 (23.4)	26 (22.8)
50.0–74.9	30 (27.0)	29 (27.1)	31 (27.2)
75.0–89.9	18 (16.2)	19 (17.8)	24 (21.1)
≥90.0	16 (14.4)	13 (12.1)	13 (11.4)
HLA classification — no. (%)†	107	106	110
Very high risk	30 (28.0)	30 (28.3)	31 (28.2)
High risk	41 (38.3)	41 (38.7)	40 (36.4)
Moderate risk	24 (22.4)	23 (21.7)	28 (25.5)
Low risk	12 (11.2)	12 (11.3)	11 (10.0)
Tanner puberty stage — no. (%)†‡	107	106	108
1	15 (14.0)	22 (20.8)	12 (11.1)
2 or 3	43 (40.2)	30 (28.3)	37 (34.3)
4 or 5	49 (45.8)	54 (50.9)	59 (54.6)
C peptide — nmol/liter†			
Fasting C-peptide	0.288±0.179	0.289±0.152	0.281±0.156
Stimulated C-peptide AUC	0.661±0.348	0.681±0.310	0.651±0.302
Glycated hemoglobin — (%)†	7.10±1.20	6.98±1.05	7.19±1.21
Insulin dose — IU/kg of body weight‡	0.539±0.276	0.599±0.345	0.568±0.284
Fasting plasma glucose — mmol/liter†	6.311±2.053	6.276±2.053	6.593±2.179
Median GAD65 autoantibody — units/ml	199.2	153.4	237.4

* Plus–minus values are means ±SD. Data are for the safety population (all enrolled patients) unless otherwise indicated. To convert values for C peptide to nanograms per milliliter, divide by 0.333. To convert values for glucose to milligrams per deciliter, divide by 0.05551. AUC denotes area under the curve, BMI body-mass index, and GAD65 the 65-kD isoform of glutamic acid decarboxylase.
† Data are for the modified intention-to-treat population.
‡ The Tanner puberty stage ranges from 1 to 5, with higher stages indicating more developed genitalia.

18.3%, 11.2%, and 21.6%; for the 12-to-15-year age group, the percentages were 47.7%, 57.0%, and 55.0%, respectively.

PRESPECIFIED EFFICACY END POINTS

Stimulated C-peptide levels showed a progressive decline from baseline to month 15 in all three study groups (Fig. 2A). As shown in Table 2, at 15 months, the treatment effect of the four-dose and two-dose regimens combined was not significantly larger than the effect of placebo ($P=0.10$). The P values for the four-dose and two-dose regimens as compared separately with the placebo group were 0.13 and 0.20, respectively. At 15 months, there were no significant differences between the four-dose and two-dose groups, compared in combination or individually with placebo, in the treatment effect on the mean daily insulin dose, glycated hemoglobin value, or any other secondary outcome (Table 2).

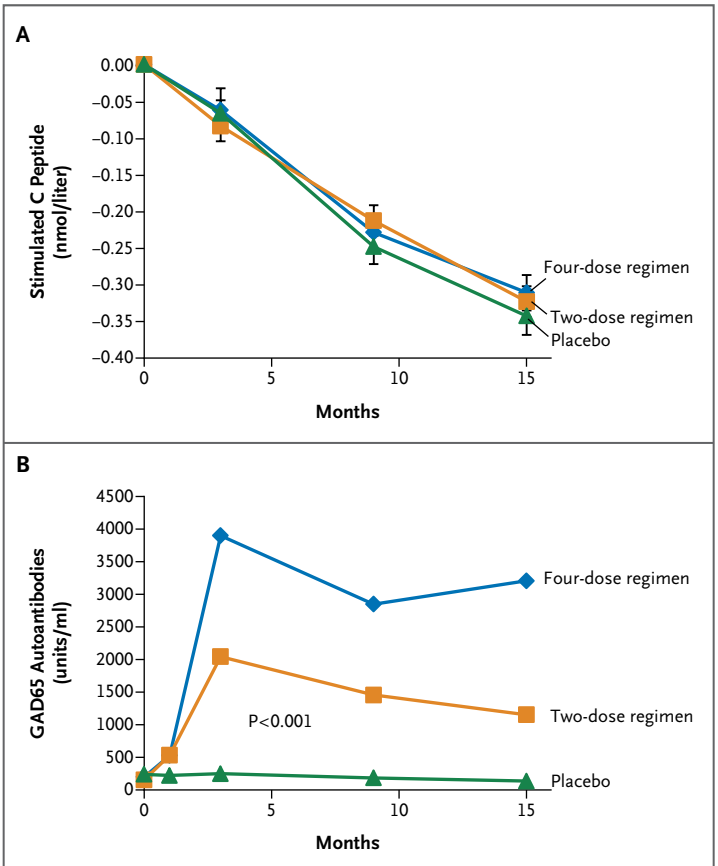

Figure 2. C-Peptide and GAD65 Autoantibody Levels, According to Study Group.

Mean changes in stimulated C-peptide levels are shown in Panel A, and median GAD65 autoantibody levels are shown in Panel B. In Panel A, the I bars indicate standard deviations. In Panel B, the two-sided P value is shown for the four-dose regimen and the two-dose regimen combined as compared with placebo. To convert values for C-peptide to nanograms per milliliter, divide by 0.333.

EXPLORATORY ANALYSES

Our prespecified subgroup analysis showed a significant effect of GAD-alum therapy in four subgroups. In the subgroup of 173 male patients, the estimated treatment ratios (i.e., the treatment effects) for the combined four-dose and two-dose groups, the four-dose group alone, and the two-dose group alone versus placebo were 1.41, 1.42, and 1.41, with P values of 0.009, 0.02, and 0.03, respectively. Among the 110 patients with a baseline Tanner pubertal stage of 2 or 3, the estimated treatment ratio for the four-dose regimen versus placebo was 1.52 (P=0.04). The 110 patients with a baseline daily insulin dose of 0.398 to 0.605 IU per kilogram of body weight had estimated treatment ratios of 1.33 for the four-dose and two-dose regimens combined (P=0.049 for the comparison with placebo) and 1.56 for the four-dose regimen alone (P=0.008 for the comparison with placebo). The estimated treatment ratio for the four-dose regimen versus placebo was 1.36 (P=0.04) among the 154 patients from non-Nordic countries (France, Germany, Italy, the Netherlands, Slovenia, Spain, and the United Kingdom).

The treatment effects for all prespecified exploratory subgroup analyses are presented for the combined four-dose and two-dose regimens (Fig. 3). (The complete subgroup analysis for male and female patients is presented in the Supplementary Appendix, available at NEJM.org.)

IMMUNE-SYSTEM EFFECTS

GAD-alum treatment resulted in a rapid rise in GAD65 autoantibody levels in both active-treatment groups at 3 months (Fig. 2B), followed by a decline at the 9-month visit; at 15 months, there was an increase in the levels in the four-dose group but a continued decline in the two-dose group. GAD65 autoantibody levels in the placebo group remained stable throughout the trial. For all time points after study-drug dosing, GAD65 autoantibody levels were significantly higher in the four-dose and two-dose groups combined than in the placebo group (P<0.001).

SAFETY

The proportion of patients with any adverse event or serious adverse event was similar between the two active-treatment groups and the placebo group (see the Supplementary Appendix). One patient in the four-dose group and one patient in the placebo group discontinued the study regimen as a result of an adverse event, but these events were deemed unlikely to be related to the study treatment. Patients with the stiff person syndrome have been shown to have elevated levels of GAD65 autoantibodies.[30] All our patients underwent neurologic assessments during the trial; there were no reported neurologic symptoms suggestive of the stiff person syndrome. Injection-site reactions were mild and similar in number among all three study groups.

DISCUSSION

Our study showed that GAD-alum treatment did not result in a significant benefit with respect to

Table 2. Prespecified Efficacy Outcomes, According to Study Group.*

Outcome	Four-Dose Regimen	Two-Dose Regimen	Combined Four- and Two-Dose Regimens	Placebo
Change in stimulated C peptide AUC from baseline, vs. placebo				
At 15 mo				
Estimated treatment ratio (95% CI)	1.180 (0.955 to 1.458)	1.149 (0.929 to 1.421)	1.164 (0.969 to 1.399)	
P value	0.13	0.20	0.10	
At 9 mo				
Estimated treatment ratio (95% CI)	1.087 (0.913 to 1.295)	1.096 (0.920 to 1.306)	1.092 (0.938 to 1.270)	
P value	0.35	0.30	0.26	
At 3 mo				
Estimated treatment ratio (95% CI)	1.025 (0.919 to 1.143)	0.985 (0.882 to 1.099)	1.005 (0.914 to 1.104)	
P value	0.66	0.79	0.92	
Change in fasting C peptide between baseline and 15 mo, vs. placebo				
Estimated treatment ratio (95% CI)	1.166 (0.940 to 1.447)	1.210 (0.974 to 1.502)	1.188 (0.985 to 1.432)	
P value	0.16	0.08	0.07	
Change in total daily insulin dose from baseline to 15 mo, vs. placebo				
Estimated treatment difference	–0.026 (–0.105 to 0.052)	–0.012 (–0.091 to 0.066)	–0.019 (–0.087 to 0.048)	
P value	0.51	0.75	0.57	
Change in glycated hemoglobin from baseline to 15 mo, vs. placebo				
Estimated treatment difference	–0.197 (–0.572 to 0.178)	0.254 (–0.123 to 0.631)	0.029 (–0.297 to 0.354)	
P value	0.30	0.19	0.86	
Change in both insulin dose and glycated hemoglobin from baseline to 15 mo, vs. placebo				
Estimated treatment difference	–0.312 (–0.861 to 0.236)	0.180 (–0.370 to 0.729)	–0.066 (–0.541 to 0.409)	
P value	0.26	0.52	0.78	
Change in maximum stimulated C peptide from baseline to 15 mo, vs. placebo				
Estimated treatment difference	0.066 (–0.007 to 0.139)	0.032 (–0.041 to 0.105)	0.049 (–0.014 to 0.112)	
P value	0.08	0.39	0.13	
Maximum stimulated C peptide >0.2 nmol/liter at 15 mo — no./total no. (%)	70/105 (66.7)	71/105 (67.6)		66/107 (61.7)
Glycated hemoglobin <7% at 15 mo — no./total no. (%)	41/106 (38.7)	31/105 (29.5)		38/105 (36.2)
Any hypoglycemic event — no./mo/patient	8.21±8.41	6.93±6.02		7.85±7.84
Severe hypoglycemic event				
No./mo/patient	0.12±0.39	0.12±0.35		0.15±0.60
Patients with[3]≥1 event — no./total no. (%)	31/109 (28.4)	30/107 (28.0)		31/110 (28.2)

* Plus–minus values are means ±SD. CI denotes confidence interval.

the change in stimulated C-peptide secretion from baseline to 15 months among patients with newly diagnosed type 1 diabetes. In addition, there was no significant benefit with respect to any of the prespecified secondary outcomes, including a change in the mean daily insulin dose or glycated

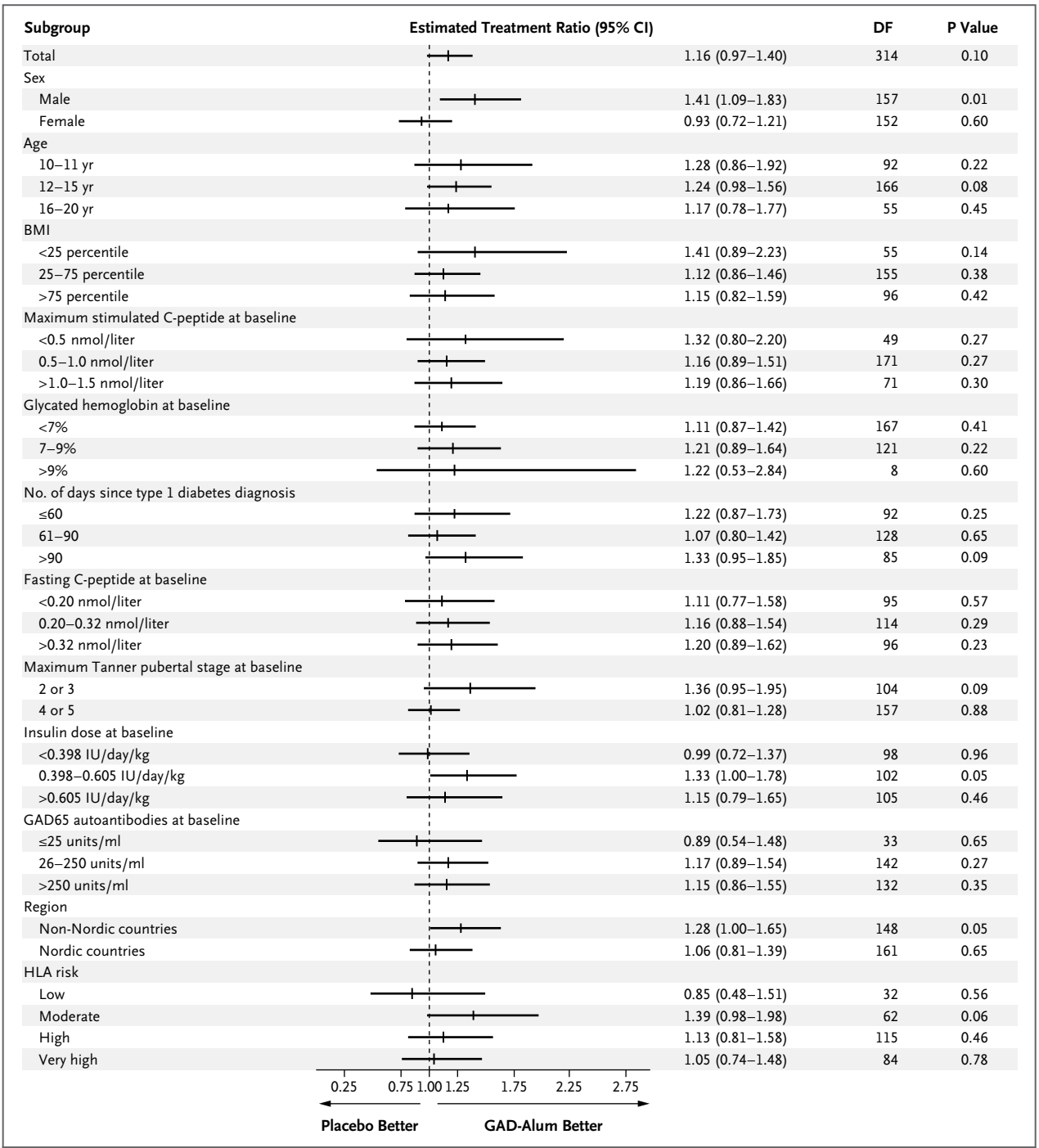

Figure 3. Estimated Treatment Ratios in Prespecified Subgroups, for the Four-Dose Group and the Two-Dose Group Combined.
To convert values for C-peptide to nanograms per milliliter, divide by 0.333. BMI denotes body-mass index, and DF degrees of freedom.

hemoglobin value. Adverse-event rates were similar among all three study groups. No neurologic symptoms suggestive of the stiff person syndrome were noted.

Exploratory analyses did not show random positive or negative effects of GAD-alum treatment, possibly owing to the multiple comparisons, yet suggesting that some subgroups may have a

response to GAD-alum treatment. Why did this phase 3 study show a lack of efficacy, in contrast to our previous phase 2 study?

One reason for the lack of efficacy in the current trial may be differences in populations or the larger numbers of clinicians with possibly different approaches to conventional treatment. On the basis of nonprespecified exploratory analyses (see the Supplementary Appendix), we speculate that seasonal variations in the immune system may play a role, since treatment seemed to have an effect in patients who received their initial GAD-alum dose in March or April, which is when all patients in the previous phase 2 trial received their treatment.[31] During the current study, an influenza epidemic occurred and resulted in widespread vaccination, which also may have influenced our results. Since the decline in C-peptide level may be more rapid in younger patients than in older patients, it may be important that our two active-treatment groups had more patients who were 10 to 11 years of age than the placebo group did, whereas the placebo group had more patients 16 to 20 years of age.[32] The incidence of type 1 diabetes is higher in boys than in girls after the age of 15 years; therefore, there may also be a difference in the autoimmune process and response to treatment according to sex.[33]

In conclusion, treatment with alum-formulated GAD65 as compared with placebo did not significantly affect the primary outcome at 15 months. Much as treatments for diseases such as childhood cancer and immunotherapy of allergy have developed in a stepwise, gradual manner through the combination of existing therapies, treatment for type 1 diabetes will most likely be based on the knowledge gained from this and other studies, as well as future studies, of single agents or combination therapies for both intervention and prevention.[34] Before autoantigen treatment is used, the dose, route, and regimen that could induce tolerance need to be better understood.[35]

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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