Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial

*Diane K Wherrett, Brian Bundy, Dorothy J Becker, Linda A DiMeglio, Stephen E Gitelman, Robin Goland, Peter A Gottlieb, Carla J Greenbaum, Kevan C Herold, Jennifer B Marks, Roshanak Monzavi, Antoinette Moran, Tihamer Orban, Jerry P Palmer, Philip Raskin, Henry Rodriguez, Desmond Schatz, Darrell M Wilson, Jeffrey P Krischer, Jay S Skyler, and the Type 1 Diabetes TrialNet GAD Study Group**

Summary

Background **Glutamic acid decarboxylase (GAD) is a major target of the autoimmune response that occurs in type 1 diabetes mellitus. In animal models of autoimmunity, treatment with a target antigen can modulate aggressive autoimmunity. We aimed to assess whether immunisation with GAD formulated with aluminum hydroxide (GAD-alum) would preserve insulin production in recent-onset type 1 diabetes.**

Methods **Patients aged 3–45 years who had been diagnosed with type 1 diabetes for less than 100 days were enrolled from 15 sites in the USA and Canada, and randomly assigned to receive one of three treatments: three injections of 20 μg GAD-alum, two injections of 20 μg GAD-alum and one of alum, or 3 injections of alum. Injections were given subcutaneously at baseline, 4 weeks later, and 8 weeks after the second injection. The randomisation sequence was computer generated at the TrialNet coordinating centre. Patients and study personnel were masked to treatment assignment. The primary outcome was the baseline-adjusted geometric mean area under the curve (AUC) of serum C-peptide during the first 2 h of a 4-h mixed meal tolerance test at 1 year. Secondary outcomes included changes in glycated haemoglobin A_{1c} (HbA_{1c}) and insulin dose, and safety. Analysis included all randomised patients with known measurements. This trial is registered with ClinicalTrials.gov, number NCT00529399.**

Findings **145 patients were enrolled and treated with GAD-alum (n=48), GAD-alum plus alum (n=49), or alum (n=48). At 1 year, the 2-h AUC of C-peptide, adjusted for age, sex, and baseline C-peptide value, was 0·412 nmol/L (95% CI 0·349–0·478) in the GAD-alum group, 0·382 nmol/L (0·322–0·446) in the GAD-alum plus alum group, and 0·413 nmol/L (0·351–0·477) in the alum group. The ratio of the population mean of the adjusted geometric mean 2-h AUC of C-peptide was 0·998 (95% CI 0·779–1·22; p=0·98) for GAD-alum versus alum, and 0·926 (0·720–1·13; p=0·50) for GAD-alum plus alum versus alum. HbA_{1c}, insulin use, and the occurrence and severity of adverse events did not differ between groups.**

Interpretation **Antigen-based immunotherapy therapy with two or three doses of subcutaneous GAD-alum across 4–12 weeks does not alter the course of loss of insulin secretion during 1 year in patients with recently diagnosed type 1 diabetes. Although antigen-based therapy is a highly desirable treatment and is effective in animal models, translation to human autoimmune disease remains a challenge.**

Funding **US National Institutes of Health.**

Lancet 2011; 378: 319–27

Published Online June 27, 2011 DOI:10.1016/S0140-6736(11)60895-7

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*Members are listed in the webappendix

Hospital for Sick Children, University of Toronto, Toronto, ON, Canada (D K Wherrett MD); **University of South Florida, Tampa, FL, USA** (B Bundy PhD, Prof J P Krischer PhD); **University of Pittsburgh, Pittsburgh, PA, USA** (Prof D J Becker MD); **Indiana University School of Medicine, Indianapolis, IN, USA** (L A DiMeglio MD, Prof H Rodriguez MD); **University of California San Francisco, San Francisco, CA, USA** (Prof S E Gitelman MD); **Columbia University, New York, NY, USA** (Prof R Goland MD); **University of Colorado Barbara Davis Center for Childhood Diabetes, Aurora, CO, USA** (Prof P A Gottlieb MD); **Benaroya Research Institute, Seattle, WA, USA** (C J Greenbaum MD); **Yale University School of Medicine, New Haven, CT, USA** (Prof K C Herold MD); **Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL, USA** (Prof J B Marks MD, Prof J S Skyler MD); **Children's Hospital Los Angeles, Los Angeles, CA, USA** (R Monzavi MD); **University of Minnesota, Minneapolis, MN, USA** (Prof A Moran MD); **Joslin Diabetes Center, Boston, MA, USA** (T Orban MD); **University of Washington School of Medicine, Seattle, WA, USA** (J P Palmer MD); **University of Texas Southwestern Medical School, Dallas, TX, USA** (Prof P Raskin MD); **University of Florida, Gainesville, FL, USA** (Prof D Schatz MD); **and Stanford University, Stanford, CA, USA** (Prof D M Wilson MD)

Introduction

Type 1 diabetes mellitus is an immune-mediated disease in which insulin-producing β cells are destroyed, resulting in lifelong dependence on exogenous insulin.[1] At the time of diagnosis, some β cells remain and their function can be measured by stimulated C-peptide responses to a mixed meal.[2] Persistence of endogenous insulin secretion, as shown by stimulated C-peptide concentrations of more than 0·2 nmol/L, has been associated with reduced occurrence of nephropathy, retinopathy, and severe hypoglycaemia.[3,4] Interventions that stop or delay the loss of C-peptide could therefore reduce the risk of diabetes complications.

Several clinical trials of interventions to delay the loss of β cells have been completed in patients with recently diagnosed type 1 diabetes. In trials of ciclosporin,[5,6] a modified anti-CD3 antibody,[7,8] rituximab,[9] and abatacept,[10] rate of loss of C-peptide has been reduced for at least the first 6 months after treatment, with raised C-peptide concentrations at 1–2 years after onset. However, the drugs in these trials might have a generalised effect on parts of the immune system and might increase risk of immunosuppression or cytokine release syndrome. Therefore, a more specific approach is highly desirable.

One such approach is to interfere with the interaction between pathogenic T cells and their target antigens. Such disruption has been achieved successfully in animal models through antigen delivery by several routes.[11] Immunisation with target antigens might promote a regulatory immune response, resulting in downregulation of autoimmunity or deletion of autoaggressive

Correspondence to:
Dr Jay S Skyler, Diabetes Research Institute, University of Miami Miller School of Medicine, PO Box 016960 (D-110), Miami, FL 33136, USA
jskyler@miami.edu

For the **study protocol** see http://www.diabetestrialnet.org/researchers/protocols.htm

antigen-specific T cells. Glutamic acid decarboxylase (GAD) has long been recognised as a target antigen in type 1 diabetes.[12] Treatment with GAD in non-obese diabetic mice, a model of type 1 diabetes, can prevent diabetes when given before the development of hyperglycaemia.[13,14] GAD formulated with aluminum hydroxide (alum), an adjuvant often used in vaccines, was used in a dose-finding study of latent autoimmune diabetes in adults.[15] A 20 µg dose given at baseline and at 4 weeks resulted in some evidence of preserved insulin secretion. However, in a subsequent study of patients with type 1 diabetes receiving the same dose and schedule,[16] decline in stimulated C-peptide seemed to be slower in those treated within 6 months of diagnosis than in those treated with placebo. GAD had a good safety profile in both studies. Therefore, we aimed to assess whether several injections of 20 µg GAD formulated with alum (GAD-alum) would preserve insulin production in patients with type 1 diabetes who were treated within 3 months of diagnosis. We included a third injection at 8 weeks after the second injection to assess for a possible improved response to an additional booster dose, which is given in some vaccine regimens.

Methods

Patients

See Online for webappendix

Screening, enrolment, and study visits took place at 15 TrialNet sites in the USA and Canada (webappendix). We screened patients aged 3–45 years who had been diagnosed with type 1 diabetes, according to American Diabetes Association criteria, for less then 100 days. Eligible patients had GAD-65 antibodies, and stimulated C-peptide concentrations of at least 0·2 nmol/L during a mixed meal tolerance test (MMTT) done at least 21 days after diagnosis of diabetes and within 37 days before randomisation. Patients were excluded if they had antibodies to HBsAg, hepatitis C, or HIV, or evidence of active Epstein-Barr virus infection. Full eligibility criteria are detailed in the study protocol.

This phase 2 clinical trial conformed to all applicable regulatory requirements. The protocol and consent documents were approved by appropriate independent ethics committees or institutional review boards. All participants (or parents) provided written, informed consent; in addition to their parents providing consent, participants younger than 18 years provided assent.

Randomisation and masking

In this parallel group study, patients were randomly assigned (1:1:1 ratio) to receive one of three treatments: three injections of 20 µg GAD-alum, two injections of 20 µg GAD-alum and and one of alum (placebo), or three injections of alum (placebo group). The randomisation sequence was computer generated at the TrialNet coordinating centre (Tampa, FL, USA) by permuted block randomisation, with a block size of six, and was stratified by participating site. Allocation was concealed by use of vials coded for each patient.

Randomisation occurred in a staggered fashion, with patients aged 16–45 years randomised initially. After

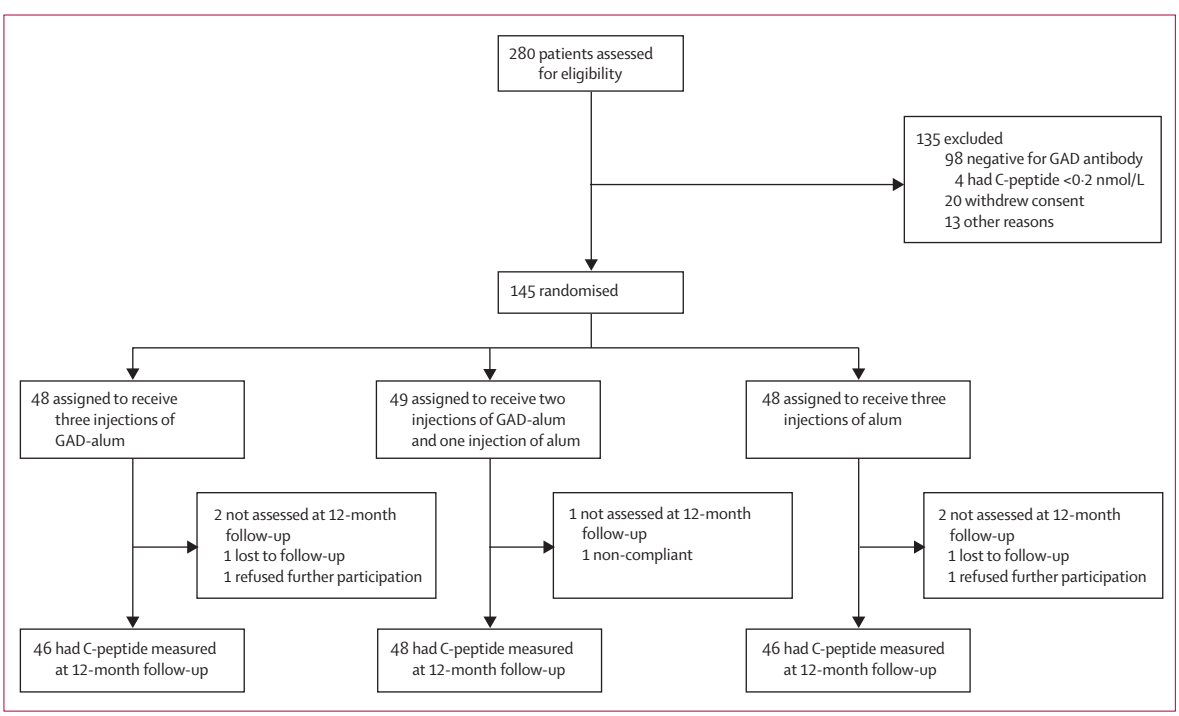

Figure 1: **Trial profile**
GAD=glutamic acid decarboxylase.

review of data from this trial and other ongoing trials of GAD-alum by regulatory agencies and the independent TrialNet data safety monitoring board (DSMB), approval was granted to randomise patients as young as 3 years. The protocol specified that a maximum of 55% of the patients could be aged 16 years or older to allow an adequate number of younger patients to be included.

Patients and clinical research personnel were masked to treatment assignment. The vials of GAD-alum and alum and their contents were indistinguishable in appearance. The DSMB reviewed interim data analyses every 6 months and did safety reviews every 3 months. An independent medical monitor (masked to treatment assignment) reviewed all accruing safety data. Adverse events were reported according to National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0).

Procedures

Every patient received three subcutaneous injections of GAD-alum or alum (Diamyd, Diamyd Medical, Stockholm, Sweden): one at baseline, one 4 weeks later, and one 8 weeks after the second injection. All patients received intensive diabetes management with the aim to achieve excellent glycaemic control, as recommended by the American Diabetes Association.[17] Every participant's primary physician retained responsibility for their diabetes management, but the research team at every study site provided support and advice. Patients used either several daily insulin injections or an insulin pump. Frequent blood glucose monitoring was done. Use of non-insulin drugs that affect glycaemic control was not allowed.

Patients were assessed by MMTTs at baseline and at 3, 6, 9, and 12 months. Patients who completed the 12-month assessment are being followed up for another 12 months, with MMTTs done every 6 months. β-cell function was assessed by stimulated C-peptide secretion. The prespecified primary outcome of this trial was a comparison of the area under the curve (AUC) of stimulated C-peptide response during the first 2 h of a 4-h MMTT done at the 12-month visit between each of the two GAD treatment groups and the placebo group.[2,18] Prespecified secondary outcomes were the slope of C-peptide over time, difference between groups in occurrence of peak C-peptide reaching less than 0·2 nmol/L, differences in glycated haemoglobin A_{1c} (HbA_{1c}) and insulin dose over time, and safety. Prespecified subgroup factors included age, sex, race, baseline C-peptide concentration, baseline insulin use, baseline HbA_{1c}, and HLA type. Additional analyses of T-cell respones and repertoires, gene expression by microarray, and cytokine responses are underway. Safety outcomes included a standardised neurological assessment because stiff person syndrome, a neurological disorder, is associated with GAD antibodies.[19]

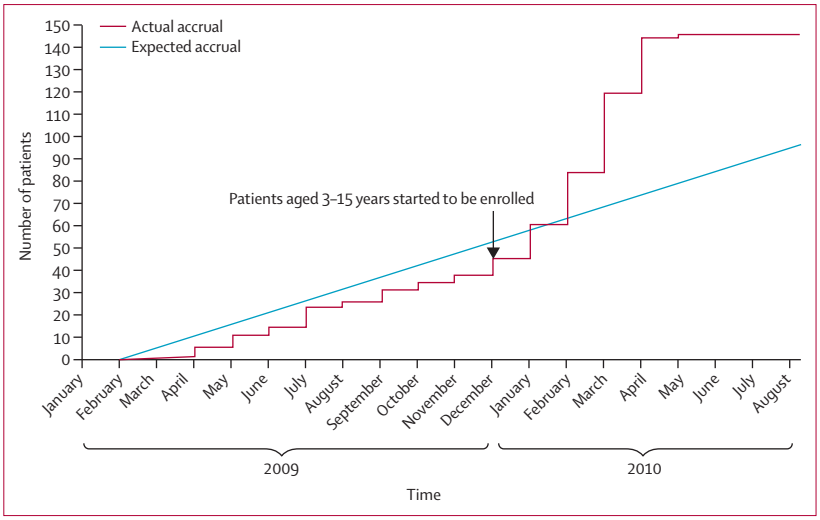

Figure 2: **Rate of enrolment**
The arrow indicated is the timepoint at which approval was granted to change the youngest age of eligible patients from 16 years to 3 years.

	Alum group (n=48)	GAD-alum plus alum group (n=49)	GAD-alum group (n=48)
Age (years)			
Mean (SD)	16·6 (9·23)	14·9 (8·72)	17·9 (10·4)
Median (range)	14·5 (4–45)	14·0 (3–45)	15·5 (3–44)
Female sex	19 (40%)	31 (63%)	14 (29%)
White race*	40 (85%)	45 (94%)	43 (91%)
Non-Hispanic ethnic origin	48 (100%)	49 (100%)	48 (100%)
Number of autoantibodies†			
1	1 (2%)	0	3 (6%)
2	10 (21%)	10 (20%)	10 (21%)
3	24 (50%)	23 (47%)	14 (29%)
4	13 (27%)	16 (33%)	21 (44%)
GAD antibody titre (index units)	0·336 (0·307)	0·349 (0·381)	0·230 (0·224)
Mean number of days from diagnosis to first infusion (SD; range)	87·1 (15·5; 42–106)	87·0 (14·1; 47–104)	83·9 (16·8; 45–105)
Weight (kg)	56·4 (21·3)	48·7 (18·4)	61·4 (22·9)
Body-mass index (kg/m^2)	20·9 (4·22)	20·0 (3·66)	22·3 (4·69)
Mean AUC of C-peptide (nmol/L)	0·690 (0·278)	0·655 (0·374)	0·710 (0·362)
HbA_{1c} (%)	6·39% (0·844)	6·63% (0·965)	6·63% (1·19)
Total daily insulin dose (U/kg)*	0·395 (0·251)	0·331 (0·219)	0·386 (0·229)
Ketoacidosis at diagnosis	6 (13%)	11 (22%)	9 (19%)
Diabetes-associated HLA alleles present*			
DR3 and DR4	14 (30%)	7 (14%)	13 (27%)
DR3 only	8 (17%)	13 (27%)	15 (31%)
DR4 only	20 (43%)	21 (43%)	13 (27%)
Neither	5 (11%)	8 (16%)	7 (15%)

Data are number of patients (%) or mean (SD), unless otherwise indicated. GAD=glutamic acid decarboxylase. HbA_{1c}=glycated haemoglobin A_{1c}. *Excludes participants with data missing for indicated variable (number missing: race, three [one in each treatment group]; insulin use, five [three in GAD-alum group, one in GAD-alum plus alum group, one in alum group]; HLA allele status, one [alum group]). †Islet cell antibodies were not tested in three patients, who were regarded as negative for the count.

Table 1: **Demographic and clinical characteristics of patients at baseline**

	Alum group	GAD-alum plus alum group		GAD-alum group	
	C-peptide (95% CI)	C-peptide (95% CI)	p value *vs* alum group	C-peptide (95% CI)	p value *vs* alum group
Unadjusted data (nmol/L)	0·418 (0·333–0·508)	0·350 (0·267–0·438)	0·75	0·448 (0·361–0·540)	0·51
Adjusted data (nmol/L)*	0·413 (0·351–0·477)	0·382 (0·322–0·446)	0·50	0·412 (0·349–0·478)	0·98
Ratio of each group to the alum group, calculated with adjusted data*	1	0·926 (0·720–1·13)	..	0·998 (0·779–1·22)	..
Loss of C-peptide from baseline	41% (1–[0·413/0·698])	42% (1–[0·382/0·662])	..	44% (1–[0·412/0·733])	..

GAD=glutamic acid decarboxylase. AUC=area under the curve. *Adjusted for age, sex, and baseline C-peptide.

Table 2: **Geometric mean 2-h AUC of stimulated C-peptide at 1 year**

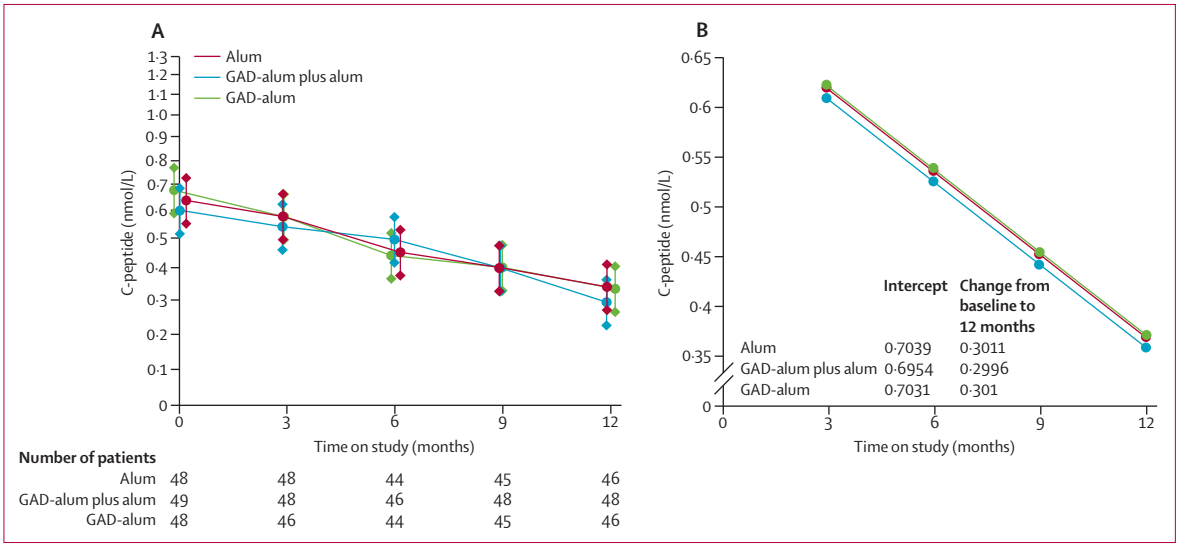

Figure 3: **C-peptide concentrations over time**
(A) Population mean of stimulated C-peptide 2-h AUC geometric mean. Estimates are from the ANCOVA model adjusted for age, sex, baseline value of C-peptide, and treatment assignment, and the y-axis is a log (y+1) scale. Error bars=95% CIs. (B) Fitted lines representing the predicted population mean of stimulated C-peptide 2-h AUC geometric mean. Estimates are from the analysis of mixed effects model adjusted for age, sex, baseline value of C-peptide, and treatment assignment, and including a fixed effect for time as a linear line on the log (y+1) scale. GAD=glutamic acide decarboxylase. AUC=area under the curve.

Blood samples were sent to TrialNet core laboratories (University of Colorado, Aurora, CO, USA; University of Washington, Seattle, WA, USA; and University of Florida, Gainesville, FL, USA) for analysis centrally. C-peptide concentrations were measured from frozen plasma by use of a two-site immunoenzymometeric assay (Tosoh Bioscience, South San Francisco, CA, USA). HbA_{1c} was measured by use of ion-exchange high performance liquid chromatography (Variant II, Bio-Rad Diagnostics, Hercules, CA, USA). Reliability coefficients for each assay were above 0·99 from split duplicate samples. Biochemical autoantibodies (microassayed insulin antibodies, GAD-65 antibodies, islet-cell antigen-512 [ICA-512] antibodies) were measured with radio-immunobinding assays, and islet-cell autoantibodies (ICA) were measured with indirect immunofluorescence. A routine chemistry panel was done with the Hitachi 917 Analyzer and reagents (Roche Diagnostics, Indianapolis, IN, USA). HLA class II alleles were measured with PCR amplification and sequence-specific hybridisation.

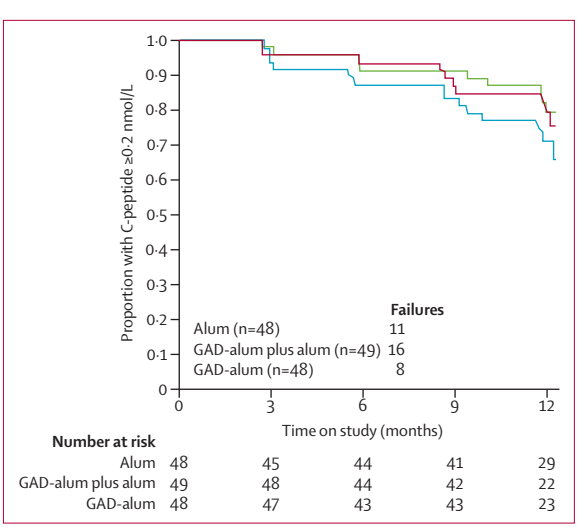

Figure 4: **Proportion of patients with 2-h peak C-peptide of 0·2 nmol/L or higher**
Data are adjusted for age, sex, and baseline value of C-peptide. GAD=glutamic acid decarboxylase.

Statistical analysis

Analyses were done with Spotfire S+ (version 8.1) for windows. All analyses were based on the prespecified modified intention-to-treat cohort of patients with known measurements; missing values were assumed to be missing at random. For simplicity, p values reported for treatment comparisons of the primary and secondary endpoints are two-sided Wald tests, although the statistical analysis plan stipulated Holm closed-sequential procedure for multiple test controlling the type I error probability at 0·05. Interim analysis for endpoint treatment effect was done and reported to the DSMB once, in accordance with the method of Lan and DeMets[20] with O'Brien-Fleming boundaries.

An ANCOVA model adjusted for age, sex, baseline value of the dependent variable, and treatment assignment was used to analyse mean AUC of C-peptide, HbA_{1c}, and total daily insulin dose. The predicted means and associated 95% CIs for each treatment group were calculated at the means of the other covariates. Significance levels associated with the treatment effect are from the Wald test (from the fitted model). A normalising transformation of log ($X_{C\text{-peptide}}$+1) was prespecified for mean AUC of C-peptide, and normal plots of the residuals indicated that this transformation was adequate. The mean AUC of C-peptide is equal to the AUC divided by the 2-h interval (ie, AUC/120). The AUC was computed by use of the trapezoidal rule from the timed measurements of C-peptide during the MMTT. The time to first stimulated peak C-peptide of less than 0·2 nmol/L (higher concentrations were associated with decreased risk of complications in the Diabetes Control and Complications Trial)[3,4,21] was analysed with standard survival methods (Cox model[22] and Kaplan-Meier[23] method). Adverse event grades were analysed with the Wilcoxon rank sum test.[24] The rate of change of mean AUC of C-peptide from 3 to 12 months was estimated by use of a mixed effects model, with both random intercept and slope adjusted for age, sex, mean AUC of C-peptide at baseline, and treatment assignment. The initial fit included a fixed interaction effect of treatment and time, but was removed because of the lack of any statistical evidence to indicate that the value was anything other than zero. To assess the treatment effect across the entire time period, a similar mixed model was fitted to the data, but time was defined without structure and was grouped by 3-month intervals.

We calculated that a sample size of 126 patients, with 42 patients per group (allocated in a 1:1:1 ratio) would be needed to provide 85% power to detect a 60% increase in geometric mean C-peptide in either GAD treatment group relative to the placebo group, with a one-sided test at α of 0·025, 10% loss to follow-up, and an expected geometric mean C-peptide of 0·248 (SD 0·179) estimated on the transformed scale log ($Y_{C\text{-peptide}}$+1).[2] Screening of new patients was closed before this target sample size was reached, but patients who had already begun screening were allowed to proceed and, thus, the sample size was exceeded.

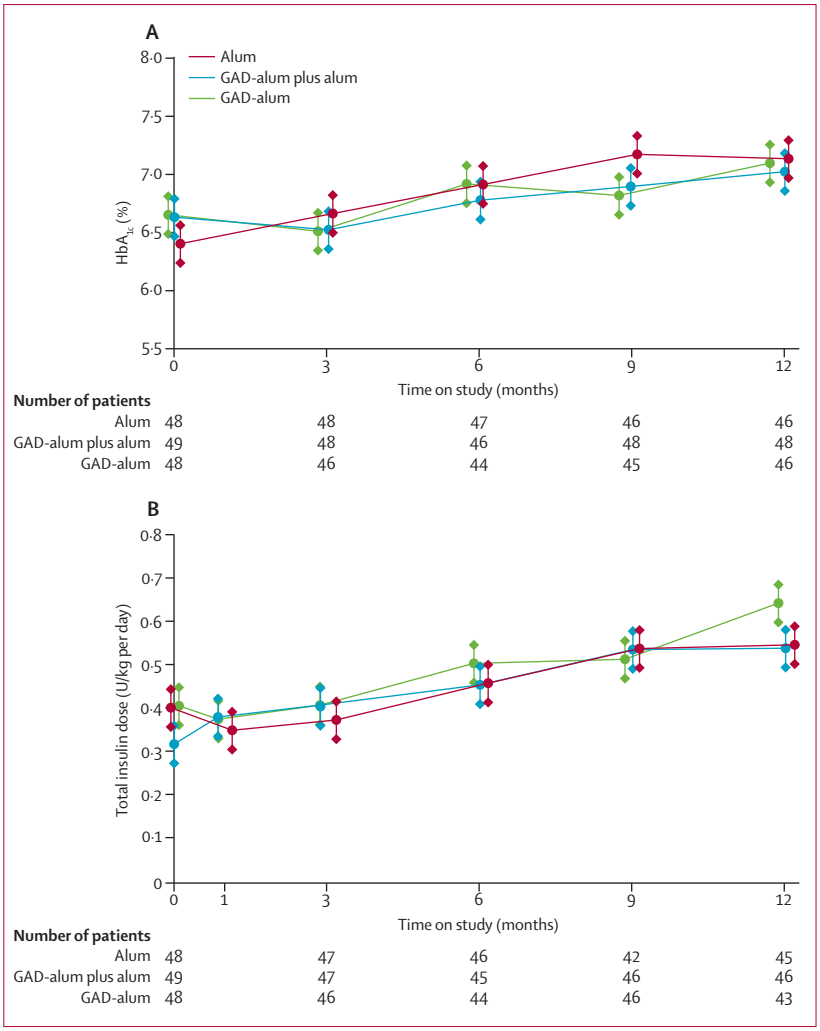

Figure 5: **HbA_{1c} and total insulin dose over time**
(A) Population mean of HbA_{1c}. (B) Population mean of insulin dose in 3-month intervals. Estimates in both A and B are from the ANCOVA model adjusted for age, sex, baseline value of HbA_{1c}, and treatment assignment. Error bars=95% CIs. GAD=glutamic acide decarboxylase. HbA_{1c}=glycated haemoglobin A_{1c}.

This trial is registered with ClinicalTrials.gov, number NCT00529399.

Role of the funding source

The trial was undertaken under the auspices of the Type 1 Diabetes TrialNet Study Group. TrialNet was responsible for the study design, data collection, data analysis, data interpretation, and writing of the report. TrialNet is funded by the US National Institutes of Health (NIH), with all members listed in the webappendix. As noted in the webappendix, some NIH staff members participated in several aspects of TrialNet, including study design. However, NIH staff did not participate in data collection, data analysis, data interpretation, or writing of the report. The writing committee (DKW, JSS, BB and JPK) had full access to all study data, and made the decision to publish the paper.

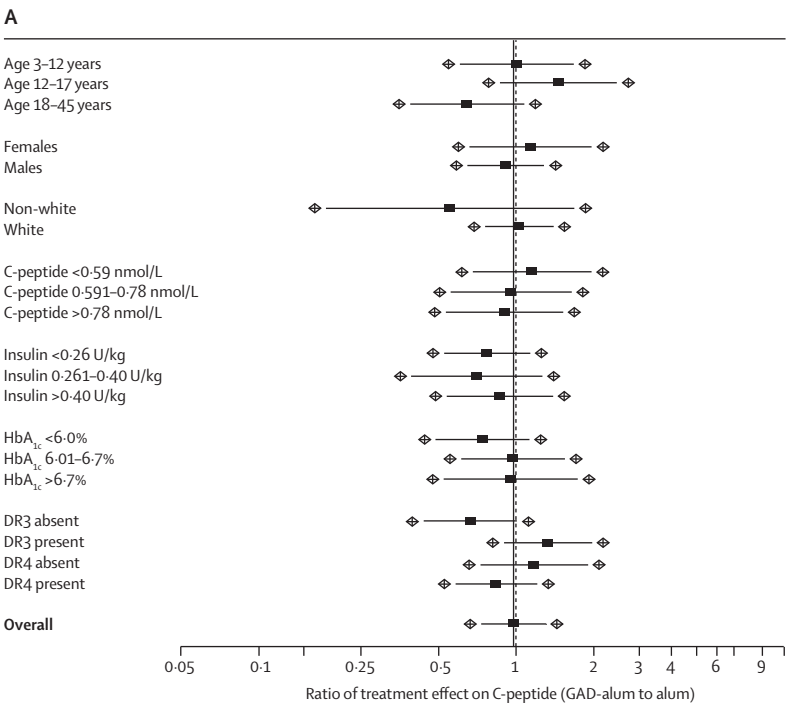

Figure 6: **Ratio of treatment effect on mean AUC of stimulated C-peptide at 12 months within categories of prespecified baseline factors**

(A) Ratio of GAD-alum to alum. The homogeneity test of treatment effect was significant for DR3 allele status (p=0·04). (B) Ratio of GAD-alum plus alum to alum. Estimates in both A and B are from the ANCOVA modelling log of C-peptide adjusted for age, sex, baseline value of C-peptide, the indicated categorised factor, treatment assignment, and treatment interaction terms. HbA_{1c}=glycated haemoglobin A_{1c}. C-peptide=geometric mean 2-h AUC of C-peptide. AUC=area under the curve. GAD=glutamic acid decarboxylase. Error bars=95% CIs.

Results

280 patients were screened for eligibility, of whom 145 (52%) were randomly assigned to receive one of three treatments (figure 1). Randomisation was done between March 23, 2009, and May 6, 2010, and the last patient completed 1-year follow-up on April 29, 2011. Rate of enrolment increased substantially after approval was granted to enrol patients aged 3–15 years (figure 2); 80 patients (55%) were younger than 16 years. Clinical and demographic characteristics were well balanced between treatment groups at baseline, with the exception that the proportion of women and girls was higher in the GAD-alum plus alum group than in the other two treatment groups (table 1).

Compliance with the protocol was excellent; 428 of 435 (98%) planned injections were received, and 140 patients (97%) had completed the MMMT at their 12-month follow-up visit by April, 2011, and were included in the primary outcome assessment. The median time delay in delivery of the injections was 0 days (IQR 0–0) from randomisation to the first injection, and was 1 day (IQR 0–5) for the second injection and 2·5 days (IQR 0–7) for the third injection from scheduled time to actual time of delivery; these delays were within the limits set out in the protocol. Delays of more than a week occurred in two patients for the first injection, five patients for the second injection, and 32 patients for the third injection. None of the delays was associated with treatment.

In the primary analysis at 1 year, the unadjusted geometric mean 2-h AUC of stimulated C-peptide did not differ significantly between the alum group and either group receiving GAD-alum (table 2). Population mean 2-h AUC of C-peptide adjusted for age, sex, and baseline C-peptide value was similar between the groups at 1 year (table 2; figure 3A). Application of the multiple imputation procedure to the five missing C-peptide values at 1 year had no effect on the significance of treatment differences. About 40% loss in mean C-peptide was recorded in all treatment groups at 1 year, but a mixed model, fitted to the C-peptide values at 3, 6, 9, and 12 months, indicated that these losses were not different between the groups, and that parallel lines properly summarised the decrease in mean C-peptide over time for each group (figure 3B). Time to stimulated peak C-peptide decreasing below 0·2 nmol/L also did not differ between the groups receiving GAD-alum and the alum group according to the Cox model with adjustment: p=0·70 for the GAD-alum group and p=0·80 for the GAD-alum plus alum group (figure 4).

HbA_{1c} and insulin dose increased gradually over time and were similar across the groups at 1 year (figure 5). At 1 year, HbA_{1c} in the alum group did not differ significantly from that in the GAD-alum group (p=0·78) or the GAD-alum plus alum group (p=0·55; figure 5A), and insulin dose in the alum group did not differ significantly from that in the GAD-alum group (p=0·10) or the GAD-alum plus alum group (p=0·89; figure 5B). Mean HbA_{1c} at

1 year was 7·07% and insulin dose was 0·527 U/kg per day in the three groups combined.

In predefined subgroup analyses we did a homogeneity test of treatment effect on age, sex, race, baseline C-peptide, insulin use, HbA_{1c}, and HLA type (figure 6). In additional analyses, treatment effect on C-peptide did not differ in individuals aged 10–18 years from those in younger and older age-groups, and baseline GAD titre (divided by tertile) did not affect treatment effect on C-peptide.

Treatment was well tolerated in all groups, with no evidence of more severe grades of adverse events in the groups receiving GAD-alum than in the group receiving alum alone (table 3). The numbers of events in the various categories of adverse events did not vary between the groups. Specifically, no symptoms suggestive of stiff person syndrome were noted. Injection site reactions did not differ between groups. Only three episodes of severe hypoglycaemia were reported as adverse events, two in the GAD-alum plus alum group and one in the alum group.

	Alum group		GAD-alum plus alum group		GAD-alum group	
	Events	Patients (n=48)	Events	Patients (n=49)	Events	Patients (n=48)
Grade of adverse events*						
None or grade 1†	..	27 (56%)	..	31 (63%)	..	30 (63%)
Grade 2	..	14 (29%)	..	13 (27%)	..	15 (31%)
Grade 3	..	5 (10%)	..	5 (10%)	..	3 (6%)
Grade 4	..	2 (4%)	..	0	..	0
Grade 5	..	0	..	0	..	0
Category of adverse events						
Allergy or immunology	0	0	0	0	2	2 (4%)
Auditory or ear	1	1 (2%)	5	3 (6%)	0	0
Blood or bone marrow	5	2 (4%)	1	1 (2%)	2	2 (4%)
Cardiac arrhythmia	0	0	1	1 (2%)	0	0
Constitutional symptoms	2	2 (4%)	0	0	0	0
Dermatology or skin	2	2 (4%)	3	2 (4%)	3	3 (6%)
Endocrine	2	2 (4%)	2	2 (4%)	2	2 (4%)
Gastrointestinal	3	3 (6%)	4	4 (8%)	3	2 (4%)
Infection	6	5 (10%)	6	4 (8%)	13	10 (21%)
Metabolic or laboratory	1	1 (2%)	0	0	0	0
Musculoskeletal or soft tissue	7	6 (13%)	1	1 (2%)	2	2 (4%)
Neurology	6	5 (10%)	0	0	0	0
Pain	1	1 (2%)	5	5 (10%)	1	1 (2%)
Pulmonary or upper respiratory	0	0	5	3 (6%)	2	2 (4%)
Renal or genitourinary	0	0	1	1 (2%)	0	0
Surgery or intra-operative injury	0	0	0	0	1	1 (2%)
Syndromes	1	1 (2%)	0	0	0	0
Total	37	..	34	..	31	..

We recorded no evidence that either of the GAD-alum treatment groups had an increased risk of severe adverse events. GAD=glutamic acid decarboxylase. *Data are for the highest grade of adverse event reported by each patient. †Grade 1 adverse events were not reportable, therefore these events are included with numbers of patients with no adverse events.

Table 3: **Adverse events**

Discussion

The results of our study show that treatment with two or three subcutaneous injections of GAD-alum, compared with alum alone, does not affect the course of loss of insulin production during 1 year in patients treated within 3 months of diagnosis of type 1 diabetes (panel). GAD antibody titres rose in the GAD-alum groups in response to the immunisations, showing that a non-protective immune response occurred. Compliance with treatment and with study outcome measurements was excellent. The number of patients randomised slightly exceeded the planned sample size, providing adequate power to answer the question posed in this study. Neither glucose control nor insulin dose varied between the groups, further supporting the lack of effect of GAD-alum. The sex imbalance between groups was probably irrelevant because modelling of the C-peptide results did not show any effect of sex on C-peptide concentrations. The study was fully enrolled in just over 1 year; enrolment was particularly rapid when approval was granted to include patients aged 3–15 years, thereby supporting the feasibility of intervention studies in recent-onset type 1 diabetes in young children.

Our study results differ from those of a previously published study of GAD-alum treatment in type 1 diabetes. In that study, patients aged 10–18 years who had been diagnosed with type 1 diabetes for less than 18 months received two injections of GAD-alum or alum.[16] The study did not meet its primary endpoint of an improvement in fasting C-peptide production at month 15, but, in a secondary analysis of patients treated within 6 months of diagnosis, concentrations of stimulated C-peptide at months 15 and 30 were significantly higher in the subgroup of 11 patients treated with GAD-alum than in the alum group of 14 patients. HbA_{1c} and insulin dose did not differ between the groups, suggesting that the apparent preserved C-peptide had little clinical effect. In view of the small sample size and multiple analyses, the findings of the subgroup analysis should probably have been interpreted as hypothesis generating but not robust. To allow a close comparison with this study, we also analysed the effect of GAD-alum treatment in the 73 patients aged 10–18 years in our study, but we recorded no effect.

Our study used the primary endpoint of stimulated C-peptide, which is more widely used than is fasting C-peptide, and was fully powered to detect a treatment effect. Concentrations of C-peptide in all study groups at baseline and 1 year were similar to the findings in the control groups of three other TrialNet studies of patients treated within 3 months of diagnosis,[9,10,26] suggesting that the results of our study are generalisable. This similarity also shows that alum alone did not have any effect on the loss of insulin secretion during 1 year.

The lack of efficacy of antigen-based therapy in our study is disappointing, but is in keeping with similar

Panel: **Research in context**

Systematic review

We searched PubMed for articles published up to May 28, 2011, with the search terms "immune intervention" and "type 1 diabetes". A comprehensive review by Luo and colleagues[25] summarised immune intervention studies done in people with type 1 diabetes. In six randomised trials with adequate sample size, treatment with ciclosporin,[5,6] modified anti-CD3 antibody,[7,8] rituximab,[9] and abatacept[10] led to some preservation of β-cell function, which was indicated by stimulated C-peptide secretion in the four most recent trials.[7–10] In a study of the GAD vaccine in type 1 diabetes, Ludvigsson and colleagues identified some suggestion of preservation of β-cell function in a small subgroup.[16]

Interpretation

In this study, two regimens of GAD vaccine (one with two doses and one with three doses), with aluminum (alum) as an adjuvant, were assessed as an antigen-based therapy for preservation of β-cell function in type 1 diabetes, as indicated by stimulated C-peptide secretion. Few adverse events occurred and treatment seemed to be well tolerated. By contrast with Ludvigsson and colleagues' study, our trial was adequately powered to assess the treatment effect, but the decline in β-cell function in patients treated with either of the two GAD vaccine regimens did not differ from that in patients treated with alum alone. Although GAD vaccine was ineffective in recent-onset diabetes, the vaccine might be beneficial for prevention of type 1 diabetes if given earlier in the course of disease, or could be a component of a combination therapy protocol in recent-onset type 1 diabetes. Clearly, however, GAD vaccine should not be used in type 1 diabetes in clinical practice.

results in trials in other autoimmune disorders, such as rheumatoid arthritis and multiple sclerosis.[27,28] Moreover, findings of trials of another diabetes-specific antigen, oral insulin, also failed to show an effect in recently-diagnosed diabetes.[29,30] Conversely, in a trial of oral insulin in relatives at risk of type 1 diabetes, a secondary analysis in a subgroup showed evidence of a potential effect;[31] a trial that is underway (NCT00419562) is further exploring this finding.[32]

Translation of successful antigen-based treatments from animal models to human disease is difficult. Findings of studies in animal models have shown that the route, dose, timing during the disease process, use of adjuvant, and frequency of antigen delivery can all affect the efficacy of tolerance induction. Successful antigen-based treatments in animal models of type 1 diabetes have most often been given before development of diabetes or at the time of development of hyperglycaemia, which is a stage earlier than in patients who had been diagnosed with type 1 diabetes for up to 3 months. In such patients, the β-cell mass is likely to be smaller and the immune response more diverse than before development of diabetes. Previous studies of GAD treatment in non-obese diabetic mice,[13,14] a model of type 1 diabetes, did not use the subcutaneous route, did not use the GAD-alum formulation for delivery of GAD, and did not use GAD after diagnosis of diabetes. GAD treatment that affects only the GAD-specific immune response might not be powerful enough to alter a mature immune response to the many β-cell antigens targeted in patients with recent onset diabetes.[33] Nonetheless, GAD treatment earlier in the course of disease could be effective, as has been shown in the mouse model.[34]

In a review, Peakman and von Herrath suggest that further research is needed to better understand the dose, route, and regimen that effectively induce tolerance.[35] This research will be greatly helped by the development of robust markers of immune regulation that could be used as measures of surrogate outcomes in these exploratory studies. Moreover, the failure to record an effect of GAD treatment alone in recent-onset type 1 diabetes does not preclude the possibility that GAD treatment might be useful as one component of a combination therapy approach that could include the use of low dose immunomodulatory agents.[36,37]

Contributors

DKW served as study chair and wrote the first draft of this report. The trial was proposed to TrialNet by JPP, who served as study vice-chair. The report writing group included DKW, JSS, BB, and JPK. All authors contributed to the conduct of the study and the collection and review of study data. The other authors reviewed and commented on various versions of the report, and suggested revisions. The members of the writing group assume responsibility for the overall content and integrity of the report.

Conflicts of interest

DKW reports receiving lecture fees from Eli Lilly and Medtronic. DJB reports receiving a grant from Diamyd. SEG reports serving on an advisory board for Genentech. RG reports receiving grants from Diamyd and Tolerx. PAG reports serving on advisory boards for Genentech, Eli Lilly, Sanofi-Aventis, and Tolerx; and reports receiving grants from Bayhill Therapeutics, Diamyd, Macrogenics, Omni BioTherapeutics, and Tolerx. CJG reports receiving grants from Bayhill Therapeutics, Diamyd, and Tolerx. JBM reports serving on an advisory board for Amgen. AM reports serving on an advisory board for Pfizer; and receiving grants from Tolerx, Merck, and Osiris Therapeutics. TO reports serving on the data safety monitoring board for Osiris Therapeutics, and being a founder of Orban Biotech. JPP reports being a consultant and receiving research grants and leading studies for Diamyd. PR reports serving on advisory boards for Amgen, AstraZeneca, MannKind, and Novo-Nordisk; serving on speakers bureaus for Merck and Novo-Nordisk; and receiving grants from Aegera, Andromeda Biotech, Bayhill Therapeutics, Biodel, Boehringer Ingelheim, Calibra, CPEX, Generex, Hoffman-LaRoche, MannKind, Novo-Nordisk, Osiris Therapeutics, and Reata. HR reports serving on an advisory board for Marcadia Biotech; serving as a consultant to Eli Lilly, Genentech, Bayer, EMD Serono, and Merck; being on the speakers bureau of Eli Lilly and Novo-Nordisk; and receiving grant support from Macrogenics and Eli Lilly. DS reports serving on advisory boards for Andromeda, Eli Lilly, GlaxoSmithKline, and Roche; giving a lecture supported by Pfizer; and receiving a grant from Diamyd. DMW reports serving on an advisory boards for DexCom and Genentech; and receiving grants support from Genentech, Diamyd, and Osiris Therapeutics. JSS reports serving on boards for Amylin Pharmaceuticals, DexCom, and Sanofi-Aventis; receiving grants from Bayhill Therapeutics, Halozyme, Intuity, and Osiris Therapeutics; receiving consultancy fees from Becton-Dickinson, Merck, MannKind Corporation, GlaxoSmithKline, Salutria Pharmaceuticals, Veroscience, Roche, and Exsulin; and receiving speakers' fees and payment for development of an educational presentation from Novo-Nordisk; and holds stock in Amylin Pharmaceuticals and Dexcom. BB, LAD, KCH, RM, and JPK declare that they have no conflicts of interest.

Acknowledgments

The sponsor of the trial was the Type 1 Diabetes TrialNet Study Group. The Type 1 Diabetes TrialNet Study Group is a clinical trials network funded by NIH through the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Allergy and Infectious Diseases, and the Eunice Kennedy Shriver National Institute of Child Health and Human Development, via cooperative

agreements (U01 DK061010, U01 DK061016, U01 DK061034, U01 DK061036, U01 DK061040, U01 DK061041, U01 DK061042, U01 DK061055, U01 DK061058, U01 DK084565, U01 DK085453, U01 DK085461, U01 DK085463, U01 DK085466, U01 DK085499, U01 DK085505, and U01 DK085509) and a contract (HHSN267200800019C); and through the National Center for Research Resources, via clinical translational science awards (UL1 RR024131, UL1 RR024139, UL1 RR024153, UL1 RR024975, UL1 RR024982, UL1 RR025744, UL1 RR025761, UL1 RR025780, UL1 RR029890, and UL1 RR031986) and a general clinical research center award (M01 RR00400). TrialNet also receives non-financial support from the Juvenile Diabetes Research Foundation International (JDRF) and the American Diabetes Association (ADA). The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of NIH, JDRF, or ADA. Diamyd Medical (Stockholm, Sweden) provided GAD-alum and placebo (alum), but had no involvement with the final study design, study conduct or management, data collection, data analysis, data interpretation, or writing of the report. There are no agreements concerning confidentiality of the data between Diamyd and the authors or the institutions named in the credit lines. The authors did provide Diamyd Medical a copy of the original report before submission. Roche Diagnostics (Indianapolis, IN, USA) provided blood glucose monitoring metres and strips to research patients. No funds were paid for preparation of this report.

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