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Antigen-Based Therapy with Glutamic Acid Decarboxylase (GAD) Vaccine in Patients with Recent-Onset Type 1 Diabetes: A Randomised Double-Masked Controlled Trial

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Abstract

Background—Type 1 diabetes (T1DM) is an autoimmune disease leading to destruction of insulin producing beta cells and life-long requirement for insulin therapy. Glutamic acid decarboxylase (GAD) is a major target of this immune response. Studies in animal models of autoimmunity have shown that treatment with a target antigen can modulate aggressive autoimmunity. We evaluated immunization with GAD formulated in aluminum hydroxide (alum) as an adjuvant in recent onset T1DM.

Methods—In this multicentre, double-masked, randomised controlled trial, 145 subjects (ages 3-45) with T1DM for less than 3 months received 3 injections of 20 µg GAD-alum (48 subjects), 2 injections of GAD-alum and one of alum alone (49 subjects) or 3 injections of alum (48 subjects) subcutaneously at baseline, 4 weeks later and 8 weeks after the second injection. Primary outcome was baseline-adjusted geometric mean 2-hour area under the curve (AUC) serum C-peptide following a mixed meal tolerance test at one year. Secondary outcomes included changes in HbA1c and insulin dose, and safety. This trial is registered in [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00529399) (NCT00529399).

Findings—The ratio (experimental to control) of the adjusted population mean of C-peptide for the GAD-alum ×3 and GAD-alum ×2/alum ×1 groups is 0.998 (95% CI: [0.779, 1.22], p = 0.98) and 0.926 (95% CI: [0.720, 1.13], p = 0.50), respectively. HbA1c and insulin use did not differ between groups. There was no difference in rate or severity of adverse events between groups.

Interpretation—Antigen-based immunotherapy therapy using GAD-alum given subcutaneously in two or three doses over 4 to 12 weeks does not alter the course of loss of insulin secretion over one year in subjects with recently diagnosed T1DM. While antigen-based therapy is a highly desirable treatment and is effective in animal models, translation to human autoimmune disease remains a challenge.

Funding—National Institutes of Health.

Keywords

glutamic acid decarboxylase; type 1 diabetes; antigen specific therapy; immune modulation; children

Type 1 diabetes mellitus (T1DM) is an immune-mediated disease in which insulin-producing beta cells are destroyed resulting in life-long dependence on exogenous insulin.¹ At the time of diagnosis, some beta 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 levels of > 0.2 nmol/L, has been associated with reduction in the rates of nephropathy, retinopathy and severe hypoglycemia.^{3,4} Interventions that stop or delay the loss of C-peptide could therefore reduce the risk of diabetes complications.

Several clinical trials using interventions to delay the loss of beta cells have been completed in recently diagnosed patients with type 1 diabetes. Trials of cyclosporine,^{5,6} of a modified anti-CD3 antibody,^{7,8} of rituximab,⁹ and of abatacept¹⁰ have shown a reduced rate of loss of C-peptide at least in the first 6 months after treatment, with higher C-peptide levels 1 to 2 years after onset. However, these trials have used drugs that may have a generalized effect on parts of the immune system and may carry potential risks either of immunosuppression or of cytokine release syndrome. Therefore, a more specific approach is highly desirable.

One such approach is to interfere with the interaction between pathogenic T-lymphocytes and their target antigens. This has been done successfully in experimental animal models through antigen administration by a number of routes.¹¹ Immunization with target antigens may promote a regulatory immune response resulting in down regulation of autoimmunity or deletion of autoaggressive antigen-specific T-lymphocytes. Glutamic acid decarboxylase (GAD) has long been recognized as a target antigen in type 1 diabetes.¹² Treatment with GAD in the non-obese diabetes mouse, a model of type 1 diabetes, can prevent diabetes when given prior to the development of hyperglycemia.^{13,14} GAD formulated with aluminum (alum), an adjuvant commonly used in vaccinations, was used in a dose-finding study in latent autoimmune diabetes in adults.¹⁵ In that study, a 20 µg dose given at baseline and at four weeks resulted in some evidence of preserved insulin secretion. A further study in patients with type 1 diabetes using the same dose and schedule appeared to show a slower decline in stimulated C-peptide in those treated within 6 months of diagnosis.¹⁶ Both studies showed good safety profiles. Therefore, we hypothesized that multiple injections with 20 µg of GAD-alum would preserve insulin production in type 1 diabetes patients treated within 3 months of diagnosis. We included a third injection eight weeks after the second injection to assess for a possible improved response to an additional booster dose as given in some vaccine regimens.

Methods

Study Design and Patients

This Phase 2 clinical trial was registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00529399) (NCT00529399), and 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 of age provided assent.

We screened 280 subjects (age 3–45 years) diagnosed (by American Diabetes Association criteria) with T1DM for less than 100 days. A total of 145 patients were randomized (from March 23, 2009 to May 6, 2010) who had glutamic acid decarboxylase-65 antibodies [GAD-65Ab] and stimulated C-peptide levels > 0.2 nmol/L measured during a mixed meal tolerance test (MMTT) conducted at least 21 days after diagnosis of diabetes and within 37 days of randomisation. Subjects with antibodies to hepatitis-B surface antigen, hepatitis-C, or human immunodeficiency virus or who had evidence of active EBV infection at the timing of screening for eligibility for the study were excluded from participation.

The first author (DKW) chaired the trial, which was originally proposed by JPP, and which was conducted under the auspices of the Type 1 Diabetes TrialNet Study Group. Diamyd Medical AB (Stockholm, Sweden) provided GAD-alum and placebo (alum) but had no involvement with study management, data collection, data analysis, or manuscript preparation. The study protocol is available at the Type 1 Diabetes TrialNet public website, www.diabetestrialnet.org.

Randomisation and Masking

This was a parallel group study, in which patients were randomly assigned in a 1:1:1 ratio, stratified by participating site. One hundred forty-five subjects were randomised to receive treatment with three injections of GAD-alum (48 subjects), two injections of GAD-alum and one of alum (placebo) (49 subjects) or three injections of alum (48 subjects). Randomisation was conducted centrally at the TrialNet Coordinating Center, using computer-generated permuted block randomisation, with a block size of 6. Randomisation occurred in a staggered fashion with those between 16 and 45 years of age being randomised initially. After review of data from this trial and other ongoing trials of GAD-alum by regulatory agencies and the TrialNet DSMB, approval was granted to randomise subjects as young as 3 years of age. The protocol specified that a maximum of 55% of the subjects could be 16 years of age to allow an adequate number of younger subjects to be included. Eighty subjects (55%) were younger than 16 years of age. Screening and subsequent study visits took place at 15 TrialNet sites in the United States and Canada (see On-Line Appendix).

Neither subjects nor clinical research personnel were aware of the treatment assignments. The GAD-alum and alum placebo vials and their contents were indistinguishable in appearance. An independent data and safety monitoring board (DSMB) reviewed interim data analyses every 6 months and conducted quarterly safety reviews. An independent medical monitor (masked to treatment assignment) reviewed all accruing safety data. Adverse events were reported using the Common Terminology Criteria for Adverse Events, version 3.0.

Procedures

GAD-alum (20 µg) or alum (Diamyd, Diamyd Medical AB, Stockholm, Sweden) was given by subcutaneous injection at baseline, four weeks later, and eight weeks after the second injection.

All subjects received intensive diabetes management. The goal was to achieve excellent glycemic control as recommended by the American Diabetes Association.¹⁷ The participants' primary physician retained responsibility for their diabetes management. The research team at each study site played a supportive and advisory role with respect to the participant's diabetes management. Patients used either multiple daily insulin injections or an insulin pump. Frequent blood glucose monitoring was performed. Usage of non-insulin pharmaceuticals that affect glycemic control was not allowed.

By April 2011, 140 out of 145 subjects (97%) had completed their 1-year visit MMTT and were included in the primary outcome assessment. Subjects continue follow up for a second year including the performance of a MMTT every 6 months.

Beta cell function was evaluated by stimulated C-peptide secretion. The pre-specified primary outcome of this trial was a comparison of the area under the curve (AUC) of stimulated C-peptide response over the first 2 hours of a 4-hour MMTT conducted at the 12 month visit between each of the two GAD treatment groups and the placebo group.^{2,18} MMTTs were performed at baseline, 3, 6, 9, and 12 months. Pre-specified secondary outcomes included: slope of C-peptide over time, difference between groups in incidence of loss of peak C-peptide to < 0.2 nmol/L, differences in HbA1c and insulin dose over time, and safety. Pre-specified subgroup factors included age, gender, race, baseline C-peptide, baseline insulin use, baseline HbA1c, and HLA type. Additional analyses of T-lymphocytes responses and repertoires, gene expression by microarray, and cytokine responses are underway.

Safety outcomes included a standardized neurological assessment because “Stiff Person Syndrome”, a neurological disorder, is associated with GAD antibodies.¹⁹

Laboratory Tests

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 levels were measured from frozen plasma using a two-site immunoenzymometric assay (Tosoh Bioscience, South San Francisco, CA, USA). HbA1c was measured using 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 (mIAA, GAD-65Ab, ICA-512Ab) were measured using radio-immunobinding assays and ICA using indirect immunofluorescence. A routine chemistry panel was performed (Roche Diagnostics, Indianapolis, IN, USA, Hitachi 917 Analyzer and reagents).

Human leukocyte antigen (HLA) class II alleles were measured using polymerase-chain-reaction amplification and sequence-specific hybridization.

Statistical Analyses

Analyses were performed using Spotfire S+® 8.1 for windows, TIBCO, Somerville, MA, USA. All analyses were based on the pre-specified intention-to-treat (ITT) cohort with known measurements. Missing values were assumed to be missing at random. For simplicity, the p-values associated with and presented for the ITT 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 conducted and reported to the DSMB once in accordance to the method of Lan and DeMets with O’Brien-Fleming boundaries.²⁰ The pre-specified analysis method for C-peptide mean AUC, HbA1c, and total daily insulin dose was an analysis of covariance model adjusting for age, gender, and baseline value of the dependent variable, and treatment assignment. The predicted means and associated 95% confidence intervals for each treatment group were determined at the means of the other covariates. The significance levels associated with the treatment effect are from the Wald test (from the fitted model). A normalizing transformation of $\log(X_{C-pep} + 1)$ was pre-specified for C-peptide AUC mean and normal plots of the residuals indicated that it was adequate. The C-peptide mean AUC equals the AUC divided by the two-hour interval (i.e. AUC/120). The AUC was computed using 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 (a level above which was associated with decreased risk of complications in the Diabetes Control and Complications Trial)^{3,4,21} was analyzed using standard survival methods (Cox Model²² and Kaplan-Meier²³ method). Adverse event grades were analyzed using Wilcoxon Rank Sum Test.²⁴ The rate of change of C-peptide mean AUC from 3 to 12 months was estimated using a mixed effects model with both random intercept and slope adjusting for age, gender, baseline C-peptide mean AUC, and treatment assignment. The initial fit included a fixed interaction effect of treatment and time but was removed due to the lack of any statistical evidence of it being other than zero. To assess the treatment effect over the entire time period, a similar mixed model was fitted to the data except time was defined without structure and grouped by three month intervals.

A sample size of 126 subjects (42 subjects per group) was planned in order to provide 85% power to detect a 60% increase in geometric mean C-peptide from either treatment relative to the placebo group using a test at the 0.025 level (one-sided), with 10% loss to follow-up and a 1:1:1 allocation to the three treatment groups (the expected mean and standard deviation estimates, on the transformed scale $\log(Y_{C-pep} + 1)$, were 0.248 and 0.179, respectively).² Screening of new subjects was closed prior to the time that this target sample size was reached; however, subjects who had already begun screening were allowed to proceed. Thus, a total of 145 subjects were randomized.

Results

Baseline Characteristics

The baseline characteristics of the three groups are summarized in Table 1. The only noteworthy imbalance was in gender with more females in the group who received two injections of GAD-alum and one of alum.

Figure 1a depicts the CONSORT diagram, showing randomisation/enrollment and follow-up of subjects during the study; Figure 1b shows the rate of enrollment.

Compliance with the protocol was excellent; 428 (of 435) (98.4%) of planned injections were received and 140 (96.6%) of subjects completed the one year primary outcome assessment. The median time delay in administering the injections was 0, 1, and 2.5 days for the first, second and third injections, respectively. These delays were within the limits set out in the protocol. Delays of more than a week occurred in 2, 5 and 32 patients, respectively. These delays were not associated with treatment.

Efficacy

In the primary analysis at one year, the geometric mean stimulated unadjusted C-peptide 2-hour AUC of the groups was: GAD-alum $\times 3$: 0.448 nmol/L (95% confidence interval: 0.361, 0.540), GAD-alum $\times 2$: 0.350 nmol/L (95% CI: 0.267, 0.438), and alum $\times 3$: 0.418 nmol/L (95% CI: 0.333, 0.508), $p = 0.51$ and 0.75 for comparison of each active treatment versus placebo. The age, gender, and baseline C-peptide adjusted population C-peptide mean 2-h AUC at 2 years was: GAD-alum $\times 3$: 0.412 nmol/L, GAD-alum $\times 2$: 0.382 nmol/L, and alum $\times 3$: 0.413 nmol/L (Figure 2a). When the multiple imputation procedure was applied to the 5 missing C-peptide values at 1 year, there was no effect on the significance level for treatment differences. The ratio (experimental to control) of the adjusted mean of the C-peptide 2-hour AUC mean for the GAD-alum $\times 3$ and GAD-alum $\times 2$ groups is 0.998 (0.412/0.413, 95% CI: [0.779, 1.22], $p = 0.98$) and 0.926 (0.382/0.413, 95% CI: [0.720, 1.13], $p = 0.50$), respectively. The loss in the mean C-peptide at one year was 44% ($1 - 0.412/0.733$), 42% ($1 - 0.382/0.662$) and 41% ($1 - 0.413/0.698$) of the baseline mean for the GAD-alum $\times 3$, GAD-alum $\times 2$, and alum $\times 3$ groups, respectively. A mixed model, fitted to the C-peptide values (3, 6, 9, and 12 month assessments), indicated that these losses were not different among the groups and that parallel lines properly summarized the decrease in mean C-peptide levels over time for each group (Figure 2b). Time to stimulated peak C-peptide falling below 0.2 nmol/L also did not differ by group based on the Cox model with adjustment, $p = 0.70$ for GAD-alum $\times 3$ and $p = 0.80$ for GAD-alum $\times 2$ (compared to alum $\times 3$) (Figure 2c).

HbA1c increased gradually over time and was similar amongst the groups at one year (GAD-alum $\times 3$: $p = 0.78$ and GAD-alum $\times 2$: $p = 0.55$) (compared to alum $\times 3$) (Figure 3a). Likewise insulin dose increased and was similar amongst groups (GAD-alum $\times 3$: $p = 0.10$ and GAD-alum $\times 2$: $p = 0.89$) (compared to alum $\times 3$) (Figure 3b). Mean HbA1c at one year was 7.07% and insulin dose was 0.527 units per kilogram in the three groups combined.

Predefined subgroup analyses were conducted; thus a homogeneity test of treatment effect was conducted on HLA type, HbA1c, insulin use, baseline C-peptide, race, gender, and age.

Results are displayed in Figure 4. In addition to these predefined subgroup analyses, we examined whether individuals age 10-18 differed from those younger and older than that group and found no differences. We also examined whether baseline GAD titre (divided by tertile) influenced outcome and again found no differences.

Safety

The treatment was well tolerated in all groups. Table 2 describes the severity and types of adverse events reported. There was no evidence of more severe grades of adverse events in the GAD treatment groups. 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. As adverse events, there were only three episodes of severe hypoglycemia reported in the study, two in the GAD-Alx2 group and one in the alum×3 group.

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 over one year in subjects treated within 3 months of diagnosis of T1DM. GAD antibody titres rose in the GAD-alum groups in response to the immunizations, demonstrating that a non-protective immune response occurred. Compliance with treatment and with study outcome measurements was excellent. The number of subjects randomised slightly exceeded the planned sample size, giving adequate power to answer the question posed in this study. Neither glucose control nor insulin dosage varied between the groups, further supporting the lack of effect of the experimental treatments. The gender imbalance between groups was likely irrelevant as modeling of the C-peptide results did not reveal any impact of gender on C-peptide levels. The study was able to fully enroll in just over one year, with particularly rapid enrollment when subjects between 3 and 15 years of age could be randomised. This supports the feasibility of carrying out studies of intervention in recent onset T1DM 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, subjects between the age of 10 and 18 with T1DM 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 did show significantly higher levels of stimulated C-peptide at month 15 and 30 in the subgroup treated with GAD-alum within 6 months of diagnosis in a secondary analysis. HbA1c and insulin dose did not differ between the groups, suggesting minimal clinical impact of the apparent preserved C-peptide. The number of subjects in the subgroup treated within 6 months of onset was 11 in the GAD-alum arm and 14 in the placebo arm. Given the small sample size and multiple analyses, it is likely that this should have been interpreted as a hypothesis-generating but not robust finding. We also analysed the effect of GAD-alum treatment in our study subjects ages 10 to 18 to allow a more close comparison with the previously published study. There was no effect of either GAD-alum treatments in the 73 of our 145 subjects who were between 10 and 18. Our study addressed the effect of GAD-alum treatment with a fully powered study and did not find a difference in the more widely used primary endpoint of stimulated C-peptide. Levels of C-peptide at baseline and at one year mirrored the findings in the control groups of three other TrialNet studies in subjects treated within 3 months of diagnosis, suggesting that our study’s results are generalisable.^{9,10,25}

This also demonstrated that alum alone did not have any impact on the loss of insulin secretion over one year.

The lack of efficacy of this study of antigen-based therapy is disappointing, but in keeping with similar results in trials in other autoimmune conditions such as rheumatoid arthritis and multiple sclerosis.^{26,27} Moreover, trials with another diabetes specific-antigen, oral insulin, also failed to show an effect in recently-diagnosed diabetes.^{28,29} On the other hand, in a trial with oral insulin in relatives at risk of type 1 diabetes, in a secondary analysis there was evidence of a potential effect in a subgroup;³⁰ this finding is being further explored in an ongoing trial.³¹

Translation of successful antigen-based treatments from animal models to human disease is difficult. Studies in animal models have shown that the route, dose, timing during the disease process, use of adjuvant, and frequency of antigen administration, can all affect the efficacy of tolerance induction. Successful antigen-based treatments in animal models of type 1 diabetes have most often been applied prior to development of diabetes or at the time of development of hyperglycemia, at a stage earlier than in patients within the first three months of the clinical diagnosis of T1DM. The beta cell mass is likely to be relatively smaller and the immune response relatively more diverse at this time. Previous studies of GAD treatment in the non-obese diabetic mouse model of type 1 diabetes did not use GAD-alum given subcutaneously after diagnosis of diabetes. It is possible that GAD treatment that affects only the GAD specific immune response may not be powerful enough to alter a mature immune response to the many beta cell antigens targeted in those with recent onset diabetes.³² Nonetheless, it is possible that such treatment earlier in the course of the disease could be effective as has been shown in the mouse model.³³ As suggested by Peakman and von Herrath in their recent review, further research needs to be done to better understand the dose, route, and regimen that effectively induce tolerance.³⁴ This research will be greatly facilitated 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 see an effect of GAD treatment by itself in recent onset type 1 diabetes does not preclude the possibility that GAD treatment may be useful as one component of a combination therapy approach that could include the use of low dose immunomodulatory agents.^{35,36}

Panel: Research in context

Systematic review

We searched the PubMed database for articles published up to May 28, 2011, with the search terms “immune intervention” AND “type 1 diabetes”. A comprehensive review by Luo et al summarised immune intervention studies performed in people with type 1 diabetes.³⁷ There have been two older^{5,6} and more four recent randomised trials⁷⁻¹⁰ with adequate sample size that have demonstrated some preservation of beta cell function in T1DM as evidenced by stimulated C-peptide secretion. These trials used cyclosporin,^{5,6} anti-CD3,^{7,8} anti-CD20,⁹ and abatacept.¹⁰ An earlier trial also evaluated GAD vaccine in T1DM and found some suggestion of preservation of beta cell function in a small subgroup.¹⁶ In contrast, our trial was adequately powered to evaluate the effect of GAD vaccine in recent onset T1DM and failed to demonstrate an effect.

Interpretation

In this study, two regimens of GAD vaccine (one with two doses, the other with three doses), using aluminum (alum) as an adjuvant, were evaluated as an antigen-based therapy to determine its effect on preservation of beta cell function in T1DM as evidenced by stimulated C-peptide secretion. There were few adverse events and the treatment appeared to be well tolerated. However, the decline in beta cell function was essentially

superimposable in subjects treated with either of the two GAD vaccine regimens and those treated with placebo containing adjuvant alone. Although GAD vaccine was ineffective in recent onset diabetes, this does not preclude it having benefit earlier in the course of the disease as a potential vaccine for T1DM prevention; nor does it preclude the possibility of it being a component of a combination therapy protocol in recent-onset T1DM. Clearly, however, GAD vaccine should not be used in T1DM in clinical practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. (A): Enrollment, Randomization, and Follow-up of Study Participants. (B): Rate of enrollment over time. Indicated is the point at which approval was granted to change the lower age for eligibility of enrollment from 16 years to 3 years.

Figure 2. (A): The population mean (and 95% confidence intervals) of stimulated C-peptide 2-hour AUC mean (expressed as nmol/L) over time for each treatment group. The estimates are from the analysis of covariance model adjusting for age, gender, baseline value of C-peptide, and treatment assignment. Y-axis is on a $\log(y + 1)$ scale. (B): The fitted line representing the population mean of stimulated C-peptide 2-hour AUC mean over time for each treatment group. The estimates are from the analysis of mixed effects model adjusting for age, gender, 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. (C): The proportion of subjects with 2 hour peak C-peptide remaining at or above 0.2 nmol/L over time for each treatment group.

Figure 3.

(A): The population mean of HbA_{1c} over time for each treatment group. The estimates are from the analysis of covariance model adjusting for age, gender, baseline value of HbA_{1c}, and treatment assignment. (B): The population mean of insulin use per kilogram of body weight over time (in 3 month interval) for each treatment group. The estimates are from the analysis of covariance model adjusting for age, gender, baseline value of HbA_{1c}, and treatment assignment.

Figure 4.

(A): The ratio (GAD-alum $\times$ 3 to alum $\times$ 3) of treatment effect on 1 year stimulated C-peptide AUC mean within categories of pre-specified baseline factors. The estimates are from the analysis of covariance modeling log of C-peptide adjusting for age, gender, baseline value of C-peptide, the indicated categorized factor, treatment assignment, and treatment interaction terms. The homogeneity test of treatment effect was significant for DR3 allele status ($p = 0.04$). (B): The ratio (GAD-alum $\times$ 2 to alum $\times$ 3) of treatment effect on 1 year stimulated C-peptide AUC mean within categories of pre-specified baseline factors. The estimates are from the analysis of covariance modeling log of C-peptide adjusting for age, gender,

baseline value of C-peptide, the indicated categorized factor, treatment assignment, and treatment interaction terms.

Table 1
Baseline Characteristics of Subjects at Entry

	AI×3 N=48	GAD/AI×2 + AI N=49	GAD/AI×3 N=48
Age- yr			
Mean	16.6 ± 9.23	14.9 ± 8.72	17.9 ± 10.4
Median	14.5	14	15.5
Range	4 - 45	3 - 45	3 - 44
Female sex- no. of patients (%)	19 (39.6)	31 (63.3)	14 (29.2)
Race - no. of patients * (%)			
White	40 (85.1)	45 (93.8)	43 (91.5)
Ethnicity - no. of patients (%)			
Non-Hispanic	48 (100.0)	49 (100.0)	48 (100.0)
No. of autoantibodies ** - no. of patients (%)			
1	1 (2.1)	0 (0.0)	3 (6.2)
2	10 (20.8)	10 (20.4)	10 (20.8)
3	24 (50.0)	23 (46.9)	14 (29.2)
4	13 (27.1)	16 (32.7)	21 (43.8)
GAD Antibody Titre (index units)	0.336 ± 0.307	0.349 ± 0.381	0.230 ± 0.224
No. of days from diagnosis to first infusion	87.1 ± 15.5	87.0 ± 14.1	83.9 ± 16.8
Range	42 - 106	47 - 104	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
AUC Mean for C-peptide (pmol/ml)	0.690 ± 0.278	0.655 ± 0.374	0.710 ± 0.362
Glycated hemoglobin at baseline (HbA1c -%)	6.39 ± 0.844	6.63 ± 0.965	6.63 ± 1.19
Total daily insulin dose at baseline * – (U/kg)	0.395 ± 0.251	0.331 ± 0.219	0.386 ± 0.229
Ketoacidosis at diagnosis - no. of patients (%)	6 (13.3)	11(25.0)	9 (21.4)
Diabetes associated HLA alleles present - no. of patients * (%)			
DR3 and DR4	14 (29.8)	7 (14.3)	13 (27.1)
DR3 only	8 (17.0)	13 (26.5)	15 (31.2)
DR4 only	20 (42.6)	21 (42.9)	13 (27.1)
Neither	5 (10.6)	8 (16.3)	7 (14.6)

Unless otherwise indicated statistics displayed are means with standard deviations.

* Descriptive statistics excludes some subjects (3 – race not reported, 3 - did not provide baseline insulin use, 1 – HLA allele test not done)

** ICA not tested on 3 subjects, (considered negative for count)

Table 2

Adverse Events. The highest grade of adverse event experienced by subjects by treatment group. There is no evidence that either experimental group had an increased risk of more severe adverse events.

Grade	Treatment Group		
	AI×3	GAD-AI×2/AI ×1	GAD-AI×3
	No. of subjects (% [*])	No. of subjects (% [*])	No. of subjects (% [*])
None or Grade 1 [*]	27 (56.2)	31 (63.3)	30 (62.5)
2	14 (29.2)	13 (26.5)	15 (31.2)
3	5 (10.4)	5 (10.2)	3 (6.2)
4	2 (4.2)	0 (0.0)	0 (0.0)
5	0 (0.0)	0 (0.0)	0 (0.0)
Total	48 (100.0)	49 (100.0)	48 (100.0)

Adverse Effect Category	Treatment Group					
	AI×3		GAD/AI×2 + AI		GAD/AI×3	
	No. of events	No. of subjects (% [*])	No. of events	No. of subjects (% [*])	No. of events	No. of subjects (% [*])
Allergy/Immunology	0	0 (0.0)	0	0 (0.0)	2	2 (4.2)
Auditory/Ear	1	1 (2.1)	5	3 (6.1)	0	0 (0.0)
Blood/BoneMarrow	5	2 (4.2)	1	1 (2)	2	2 (4.2)
CardiacArrhythmia	0	0 (0.0)	1	1 (2)	0	0 (0.0)
ConstitutionalSymptoms	2	2 (4.2)	0	0 (0.0)	0	0 (0.0)
Dermatology/Skin	2	2 (4.2)	3	2 (4.1)	3	3 (6.2)
Endocrine	2	2 (4.2)	2	2 (4.1)	2	2 (4.2)
Gastrointestinal	3	3 (6.2)	4	4 (8.2)	3	2 (4.2)
Infection	6	5 (10.4)	6	4 (8.2)	13	10 (20.8)
Metabolic/Laboratory	1	1 (2.1)	0	0 (0.0)	0	0 (0.0)
Musculoskeletal/SoftTissue	7	6 (12.5)	1	1 (2)	2	2 (4.2)
Neurology	6	5 (10.4)	0	0 (0.0)	0	0 (0.0)
Pain	1	1 (2.1)	5	5 (10.2)	1	1 (2.1)
Pulmonary/UpperRespiratory	0	0(0.0)	5	3 (6.1)	2	2 (4.2)
Renal/Genitourinary	0	0 (0.0)	1	1 (2)	0	0 (0.0)
Surgery/Intra-OperativeInjury	0	0 (0.0)	0	0 (0.0)	1	1 (2.1)
Syndromes	1	1 (2.1)	0	0 (0.0)	0	0 (0.0)
Total	37	--	34	--	31	--

* Grade 1 adverse events were not reportable, therefore, Grade 1 adverse events are included among those with no adverse events reported. Denominator in percent calculation is number in respective treatment groups