UCSF
UC San Francisco Previously Published Works

Title

Low-Dose Anti-Thymocyte Globulin (ATG) Preserves β-Cell Function and Improves HbA1c in New-Onset Type 1 Diabetes

Permalink

https://escholarship.org/uc/item/0kp8z6sz

Journal

Diabetes Care, 41(9)

ISSN

1066-9442

Authors

Haller, Michael J
Schatz, Desmond A
Skyler, Jay S
et al.

Publication Date

2018-09-01

DOI

10.2337/dc18-0494

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Low-Dose Anti-Thymocyte Globulin (ATG) Preserves β-Cell Function and Improves HbA_{1c} in New-Onset Type 1 Diabetes

Diabetes Care 2018;41:1917–1925 | https://doi.org/10.2337/dc18-0494

*Michael J. Haller,[1] Desmond A. Schatz,[1] Jay S. Skyler,[2] Jeffrey P. Krischer,[3] Brian N. Bundy,[3] Jessica L. Miller,[3] Mark A. Atkinson,[1] Dorothy J. Becker,[4] David Baidal,[2] Linda A. DiMeglio,[5] Stephen E. Gitelman,[6] Robin Goland,[7] Peter A. Gottlieb,[8] Kevan C. Herold,[9] Jennifer B. Marks,[2] Antoinette Moran,[10] Henry Rodriguez,[3] William Russell,[11] Darrell M. Wilson,[12] Carla J. Greenbaum,[13] and the Type 1 Diabetes TrialNet ATG-GCSF Study Group**

OBJECTIVE

A pilot study suggested that combination therapy with low-dose anti-thymocyte globulin (ATG) and pegylated granulocyte colony-stimulating factor (GCSF) preserves C-peptide in established type 1 diabetes (T1D) (duration 4 months to 2 years). We hypothesized that *1*) low-dose ATG/GCSF or *2*) low-dose ATG alone would slow the decline of β-cell function in patients with new-onset T1D (duration <100 days).

RESEARCH DESIGN AND METHODS

A three-arm, randomized, double-masked, placebo-controlled trial was performed by the Type 1 Diabetes TrialNet Study Group in 89 subjects: 29 subjects randomized to ATG (2.5 mg/kg intravenously) followed by pegylated GCSF (6 mg subcutaneously every 2 weeks for 6 doses), 29 to ATG alone (2.5 mg/kg), and 31 to placebo. The primary end point was mean area under the curve (AUC) C-peptide during a 2-h mixed-meal tolerance test 1 year after initiation of therapy. Significance was defined as one-sided *P* value < 0.025.

RESULTS

The 1-year mean AUC C-peptide was significantly higher in subjects treated with ATG (0.646 nmol/L) versus placebo (0.406 nmol/L) (*P* = 0.0003) but not in those treated with ATG/GCSF (0.528 nmol/L) versus placebo (*P* = 0.031). HbA_{1c} was significantly reduced at 1 year in subjects treated with ATG and ATG/GCSF, *P* = 0.002 and 0.011, respectively.

CONCLUSIONS

Low-dose ATG slowed decline of C-peptide and reduced HbA_{1c} in new-onset T1D. Addition of GCSF did not enhance C-peptide preservation afforded by low-dose ATG. Future studies should be considered to determine whether low-dose ATG alone or in combination with other agents may prevent or delay the onset of the disease.

Type 1 diabetes (T1D) is a T-cell–mediated process characterized by autoimmune destruction of β-cells and a lifelong dependence on exogenous insulin (1). To date, the overwhelming majority of efforts seeking to impede the autoimmune process and reverse hyperglycemia have utilized single immunosuppressive or immunomodulatory drugs (2–6). While several agents targeting T and B lymphocytes have shown promise, no single agent has demonstrated long-term success in preserving C-peptide or reducing HbA_{1c} as a means of standard medical practice (7,8). Combination therapy has been proposed as a potential strategy toward developing safe and practical approaches to the preservation of C-peptide in patients with TID (9–11).

[1]*University of Florida, Gainesville, FL*
[2]*Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL*
[3]*University of South Florida, Tampa, FL*
[4]*University of Pittsburgh, Pittsburgh, PA*
[5]*Indiana University, Indianapolis, IN*
[6]*University of California, San Francisco, San Francisco, CA*
[7]*Columbia University, New York, NY*
[8]*University of Colorado Barbara Davis Center for Childhood Diabetes, Aurora, CO*
[9]*Yale University, New Haven, CT*
[10]*University of Minnesota, Minneapolis, MN*
[11]*Vanderbilt University, Nashville, TN*
[12]*Stanford University, Palo Alto, CA*
[13]*Benaroya Research Institute, Seattle, WA*

Corresponding author: Michael J. Haller, hallemj@peds.ufl.edu.

Received 6 March 2018 and accepted 12 June 2018.

Clinical trial reg. no. NCT02215200, clinicaltrials.gov.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc18-0494/-/DC1.

**A complete list of the members of the Type 1 Diabetes TrialNet ATG-GCSF Study Group can be found in the Supplementary Data online.*

© 2018 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at http://www.diabetesjournals.org/content/license.

Preclinical studies with combination therapies were performed using the nonobese diabetic (NOD) mouse model. Low-dose anti-thymocyte globulin (ATG) in combination with agents capable of providing synergy was proposed as a means of reducing adverse events and improving efficacy. Notably, granulocyte colony-stimulating factor (GCSF) was shown to improve the therapeutic capacity for diabetes reversal in NOD mice when combined with low-dose murine ATG (12). In fact, combinations including GCSF afforded greater efficacy in the NOD mouse than any other single-drug or combination approach tested. Efforts to use either GCSF or higher-dose ATG (6.5 mg/kg) as monotherapy in patients with recent-onset T1D failed to preserve C-peptide, although in post hoc analysis subjects >21 years of age appeared to benefit from 6.5 mg/kg of ATG (13,14).

A randomized, placebo-controlled, single-masked pilot clinical trial of low-dose ATG (2.5 mg/kg) and pegylated GCSF (6 mg subcutaneously every 2 weeks for 6 doses) was performed in patients with established T1D (duration of T1D 4 months to 2 years). The pilot demonstrated that combination therapy with low-dose ATG/GCSF preserved C-peptide in established T1D (15,16). Mechanistic examination of blood cells from treated subjects showed that low-dose ATG/GCSF achieved a relative increase of regulatory T cells (Treg) in circulation (17). Notably, the pilot low-dose ATG/GCSF versus placebo study did not include an arm with subjects receiving low-dose ATG monotherapy.

To explore the potential of low-dose ATG/GCSF or low-dose ATG monotherapy to preserve β-cell function in new-onset T1D, the Type 1 Diabetes TrialNet Study Group conducted a three-arm, randomized, double-masked, placebo-controlled trial (low-dose ATG/GCSF, low-dose ATG, and placebo) in persons with new-onset T1D (duration of disease <100 days).

RESEARCH DESIGN AND METHODS

Study Design and Patients

This study was registered with ClinicalTrials.gov (NCT02215200) 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; participants <18 years of age signed a study assent form.

Screening and subsequent study visits took place at 14 TrialNet sites in the U.S. (Supplementary Data). We screened 113 patients (aged 12–45 years) diagnosed with T1D for <100 days. A total of 89 patients were enrolled (from December 2014 to June 2016) who had at least one T1D-related autoantibody (microinsulin autoantibodies [mIAA], tested only if duration of insulin therapy was <7 days; glutamic acid decarboxylase-65 autoantibodies [GAD-65Ab], islet cell antigen-512 autoantibodies [ICA-512Ab], zinc transporter 8 autoantibodies [ZnT8A], or islet cell autoantibodies [ICA]) and had stimulated C-peptide levels ≥0.2 nmol/L measured during a mixed-meal tolerance test (MMTT) conducted at least 21 days after diagnosis of T1D and within 37 days of randomization.

M.J.H. proposed the trial, which was conducted under the auspices of the Type 1 Diabetes TrialNet Study Group. Sanofi (Cambridge, MA) provided Thymoglobulin (ATG) but had no involvement with study management, data collection, data analysis, or manuscript preparation. Amgen (Thousand Oaks, CA) provided Neulasta (GCSF) and placebo for the study and similarly had no further involvement. Roche Diabetes Care (Indianapolis, IN) provided glucose meters, test strips, and lancets for diabetes management.

Randomization and Masking

Patients were randomly assigned in a 1:1:1 ratio, stratified by participating site, with 29 subjects randomized to receive experimental treatment with ATG and GCSF, 29 subjects randomized to receive ATG alone (and GCSF placebo), and 31 subjects randomized to receive both placebos. Randomization was conducted centrally at the TrialNet Coordinating Center.

The study was double-masked. An independent data and safety monitoring board reviewed adverse events and study accrual every 6 months and conducted quarterly safety reviews. An independent medical monitor (masked to treatment assignment) reviewed all accruing safety data.

Procedures

ATG or placebo was administered at a dose of 2.5 mg/kg as two divided intravenous infusions of 0.5 mg/kg and 2 mg/kg. Most subjects received ATG or placebo infusion as part of a 2- to 3-day hospitalization, although some subjects were managed entirely as outpatients. Premedication for ATG/placebo infusions included oral diphenhydramine 1.25 mg/kg up to 50 mg, oral acetaminophen 15 mg/kg up to 650 mg, and intravenous methylprednisolone or placebo at 0.25 mg/kg. Subjects who developed serum sickness in the 1–2 weeks following ATG infusion were offered oral prednisone with dosing and duration of therapy at the discretion of the site investigator. Subjects weighing >50 kg were offered 50 mg every 12 h on days 1–3, then 40 mg every 12 h on day 4, 30 mg every 12 h on day 5, 20 mg every 12 h on day 6, and 10 mg every 12 h on day 7. Subjects weighing <50 kg were offered 30 mg every 12 h on days 1–3, then 20 mg every 12 h on day 4, 10 mg every 12 h on day 5, and 5 mg every 12 h on days 6 and 7. GCSF or placebo was administered every 2 weeks for a total of 6 doses at a dose of 6 mg subcutaneously or, if the patient weighed <44.5 kg, a dose of 100 μg/kg. All subjects received intensive diabetes management. The goal was to achieve intensive glycemic control as recommended by the American Diabetes Association (18). Patients used either multiple daily insulin injections or an insulin pump. Frequent daily blood glucose monitoring was performed, in some cases with concomitant use of a continuous glucose monitor. Use of noninsulin pharmaceuticals that affect glycemic control was not allowed.

Laboratory Tests

Blood samples were sent to TrialNet core laboratories for central analyses. C-peptide levels were measured from frozen plasma using a two-site immunoenzymometric assay (Tosoh Bioscience, South San Francisco, CA). HbA_{1c} was measured using ion-exchange high-performance liquid chromatography (Variant II, Bio-Rad Diagnostics, Hercules, CA). Reliability coefficients for each assay were above 0.99 from split duplicate samples. Biochemical autoantibodies (mIAA, GAD-65Ab, ICA-512Ab, ZnT8A) were measured using radioimmunobinding assays, and ICA was measured using indirect immunofluorescence. A routine chemistry panel was performed (Hitachi 917 with reagents from Roche Diagnostics). Subjects who screened positive for

serum antibodies to hepatitis B surface antigen, hepatitis C, or HIV were excluded from participation. Human leukocyte antigen class II alleles were measured using PCR amplification and sequence-specific hybridization. CD4 and CD8 cell counts were measured in whole blood via an FC500 using four-color fluorescent monoclonal antibody reagents and software for automated analysis (Beckman Coulter, Indianapolis, IN).

Statistical Methods

The prespecified primary outcome of this trial was a comparison of the area under the curve (AUC) of stimulated C-peptide response over the first 2 h of a 4-h MMTT conducted at the 12-month visit. End point analyses were based on the protocol prespecified intention-to-treat cohort, defined as all participants with measured 1-year C-peptide AUC regardless of treatment compliance. By August 2017, two subjects had withdrawn from the study. As such, 87 of the 89 randomized subjects completed their 1-year visit MMTT and were included in the primary outcome assessment. The AUC mean was computed using the trapezoidal rule calculated by the weighted sum of the timed C-peptide values during the MMTT test, then divided by 120 (minutes).

The primary statistical hypotheses sought to determine whether the ATG or ATG/GCSF group AUC C-peptide means were greater than the placebo group mean. This analysis was conducted on a transformed scale using the function $\log(Y_{cp} + 1)$ to provide better normal distributional behavior by the test statistic. The comparison of either experimental treatment group with the placebo group was based on a Wald test using an ANCOVA model adjusting for sex, baseline age, and baseline C-peptide at the 0.025 α level (one-sided). The predicted means and associated 95% CI for each treatment group were determined at the means of the other covariates.

Mean rate of change of C-peptide mean AUC was estimated using a mixed-effects model with both random intercept and slope adjusting for age, sex, baseline C-peptide AUC mean, and treatment assignment.

Prespecified subgroup factors included baseline age, sex, C-peptide, HbA_{1c}, race/ethnicity, and human leukocyte antigen. Prespecified secondary outcomes included safety, slope of C-peptide over time, and differences in HbA_{1c}, glucose, and insulin dose over time.

Sample Size

Using standard equations for the comparison of two means, a sample size of 26 per group with complete data provided power of 85% to detect a 50% increase in the geometric-like mean in either experimental treatment group (compared with the control group) testing at the 0.025 α level (one-sided), assuming the control group geometric-like mean was 0.433 nmol/L and the SD (on the transformed scale) was 0.167. These estimates were based on previous TrialNet phase II studies (19). Assuming 10% dropout, the sample size goal was set at 28 per group. The overall type I error for the trial was $\sim$0.05 given two pairwise tests.

If both hypotheses were rejected, the plan was to employ a method proposed by Simon et al. (20) to select which treatment should be considered first for further clinical research, with the understanding that the adverse effect results might influence the choice.

The design included an adaptive noncomparative (i.e., the observed interim treatment effect has no influence on the re-estimation) procedure for sample size. The SD (specifically, square root of the residual mean squared error) and the mean of the control group are two parameters required to determine sample size goal and could adversely affect the statistical power if they differ from the initial values used. The plan was to check these initial values against the observed estimates halfway through the trial ($\sim$39 patients with primary end point measured). The specified procedure was to calculate a weighted average of the initial and observed estimates and determine a new sample size goal. Due to rapid recruitment, the initial target sample size (N = 84) was exceeded (N = 89 randomized) in advance of half of the cohort reaching the primary end point. Per protocol, recruitment was paused until the re-estimation procedure could be performed. The re-estimation procedure indicated a goal of 27 per group (an increase of 1 from the initial plan). Since the number of patients missing their primary end point was much less than expected, accrual was sufficient to achieve the required number and the study was formally closed to accrual.

Adverse Events

Adverse events were defined using Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (21). The number of events and number of patients experiencing events were tabulated. As prespecified, only grades 2 and higher were counted as adverse events. Only subjects who received at least one dose of active or placebo therapy were included. The Fisher exact test (22) (one-tailed) was applied to each system pair of 2 $\times$ 2 tables (each treatment vs. placebo group). Adverse event grades were analyzed using the Wilcoxon rank sum test (22). All analyses were conducted in either TIBCO Spotfire S+ 8.2 Workbench or SAS 9.4.

RESULTS

Patient Enrollment

A total of 113 subjects were screened for eligibility, of whom 89 were randomized to one of three treatment arms: ATG/GCSF, ATG only, or placebo (Fig. 1). Clinical and demographic characteristics were similar between treatment groups (Table 1). Compliance with the study protocol was high (Supplementary Tables 1 and 2 and Supplementary Fig. 1*A*–*C*). One subject was randomized but withdrew consent prior to receiving any study drug. All remaining participants received ATG or placebo per protocol. Ninety percent of all subjects received all 6 doses of GCSF or placebo subcutaneous injections. Of the 511 doses of GCSF or placebo that were administered, only 9 involved reduced doses per protocol: 5, 1, and 3 for the ATG/GCSF, ATG only, and placebo groups, respectively. All but two subjects had AUC C-peptide assessments completed at the 12-month visit. As prespecified, these two were not included in the analysis.

C-Peptide

At 1 year, AUC C-peptide was higher in subjects treated with low-dose ATG versus placebo (P = 0.0003) (Fig. 2*A*). In those receiving ATG/GCSF, the AUC C-peptide was not significantly different from placebo (P = 0.031, with significance defined as $P < 0.025$ to adjust for multiple comparisons). At 1 year, the AUC C-peptide geometric-like means were ATG/GCSF 0.528 nmol/L (95% CI 0.435, 0.627), ATG only 0.646 (0.547, 0.750),

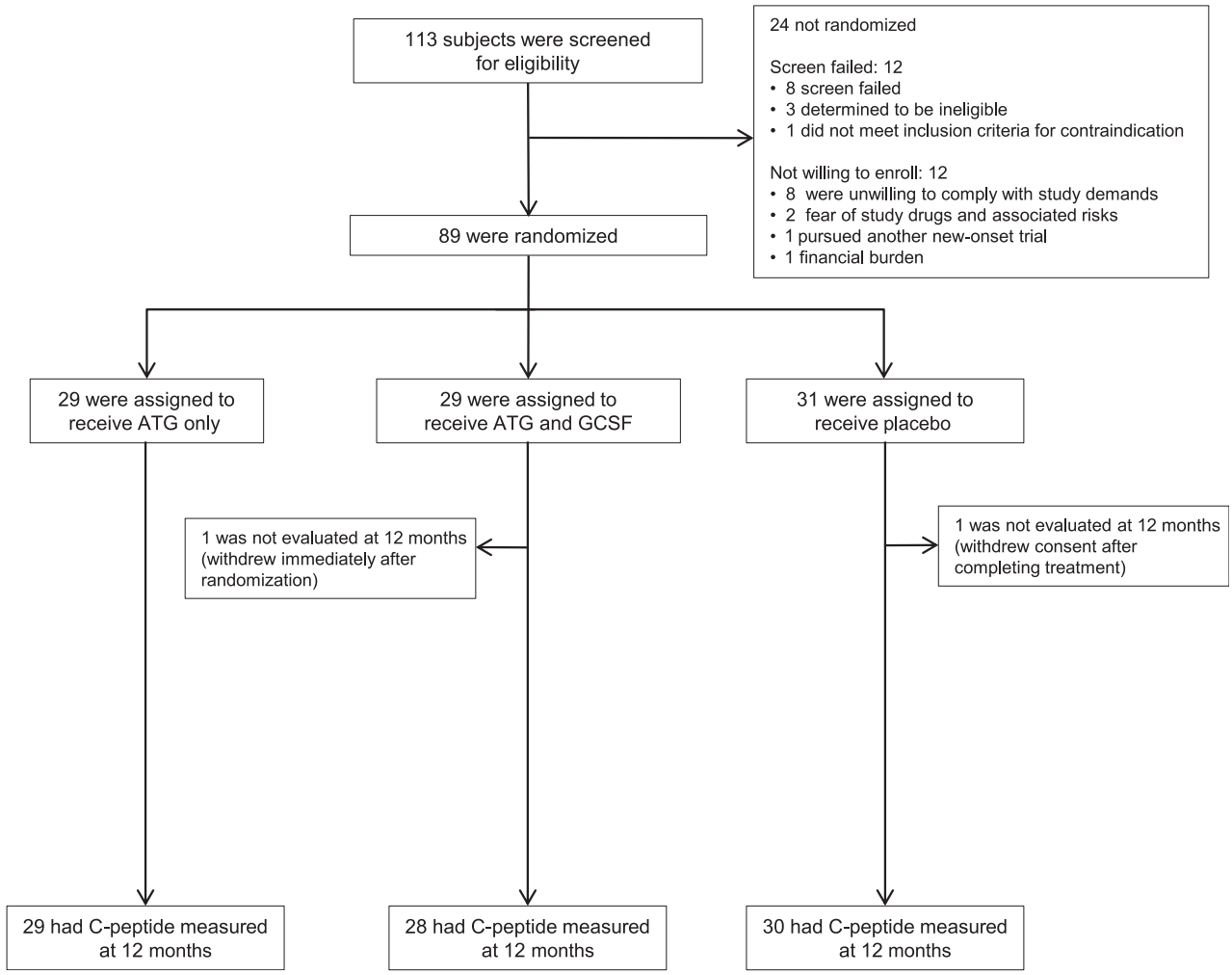

Figure 1—Consort diagram. A total of 113 subjects were screened for eligibility, of whom 89 were randomized and 87 completed the primary outcome measure at 1 year. One subject was randomized but withdrew consent prior to receiving any study drug. All remaining participants received ATG/placebo infusions as specified in the protocol. This included two participants who received reduced doses per protocol specifications (one ATG and one placebo).

and placebo 0.406 (0.324, 0.494). The C-peptide responses within treatment groups were heterogeneous, as shown in spaghetti plots of individual AUC C-peptide levels (Supplementary Fig. 2*A*–*C*).

A mixed model was employed to fit all the C-peptide measurements through 12 months (Fig. 2*B*). Consistent with the primary analysis by ANCOVA, the rates of decline for both experimental treatment groups were less than for the placebo group but not statistically different from each other. The difference in the rate of decline for the ATG/GCSF and ATG only groups (relative to the placebo group) was +0.178 and +0.227 nmol/L per year, respectively. AUC C-peptide values adjusted to show equivalent baseline values and change over time in the treatment groups are shown in Supplementary Fig. 3. AUC glucose during the 2-h MMTT was lower in subjects treated with ATG/GCSF and ATG alone than in placebo-treated subjects (Supplementary Fig. 4).

HbA_{1c}

At 1 year following therapy, HbA_{1c} was significantly lower in both experimentally treated groups versus placebo: ATG/GCSF versus placebo $P = 0.011$ and ATG only versus placebo $P = 0.002$ (Fig. 2*C*). HbA_{1c} was adjusted for baseline HbA_{1c} level, age, and sex using ANCOVA.

Insulin Use

There were no statistically significant differences in insulin use between either experimental treatment group or the placebo group (Fig. 2*D*). Reported insulin use (units/kg/day) was adjusted for baseline insulin use, age, and sex by ANCOVA. There were three patients who did not have baseline insulin use reported; the mean level at baseline was imputed for these patients in order that they could be included.

Lymphocyte and CD4/CD8 Ratio

Patients who received low-dose ATG or low-dose ATG/GCSF experienced reduced total lymphocytes, reduced CD4 T cells, and relative preservation of CD8 T cells, resulting in a reduction in the CD4/CD8 ratio in comparison with subjects who received placebo (Fig. 2*E* and Supplementary Fig. 5). Complete blood counts with differential data are provided in Supplementary Table 3.

Adverse Events

Adverse events separated by severity (grade) and adverse event category are shown in Tables 2 and 3, respectively. Events of grade 2 or higher were counted as adverse events per protocol. In all analyses of adverse events, the one patient who withdrew prior to receiving

Table 1—Baseline characteristics

Patient characteristics	ATG and GCSF (*N* = 29)	ATG only (*N* = 29)	Placebo (*N* = 31)
Age, years			
Mean ± SD	17.2 ± 5.0	18.1 ± 6.9	16.9 ± 4.6
Median	16.4	15.5	15.0
Range	12.0–32.8	12.4–42.5	12.2–29.3
Male sex, no. of patients (%)	16 (55.2)	17 (58.6)	17 (54.8)
Race, no. of patients (%)			
White	28 (96.6)	29 (100.0)	29 (93.5)
Black	1 (3.4)	0 (0.0)	2 (6.5)
Ethnicity not Hispanic or Latino, no. of patients (%)	28 (96.6)	27 (93.1)	30 (96.8)
Autoantibodies positive, no. of patients (%)			
GAD65H	23 (79.3)	23 (79.3)	23 (74.2)
IA2H	25 (86.2)	23 (79.3)	25 (80.6)
ICA*	26 (92.9)	25 (86.2)	22 (71.0)
ZNT8	21 (72.4)	21 (72.4)	22 (71.0)
No. of autoantibodies positive, no. of patients (%)*			
1	0 (0.0)	3 (10.3)	1 (3.2)
2	3 (10.3)	2 (6.9)	3 (9.7)
3	2 (6.9)	1 (3.4)	5 (16.1)
4	11 (37.9)	11 (37.9)	13 (41.9)
5	13 (44.8)	12 (41.4)	9 (29.0)
No. of days from diagnosis to randomization			
Median	83	81	84
Range	49–97	47–100	52–99
Weight, kg			
Median	62.3	66.4	62.0
Range	39.8–89.1	39.6–92.4	33.8–118
BMI, kg/m^2			
Median	21.4	22.6	21.8
Range	16.6–27.7	15.2–32.8	14.3–34.3
AUC mean for C-peptide, nmol/L			
Mean ± SD	0.793 ± 0.321	0.878 ± 0.474	0.966 ± 0.503
Median	0.701	0.757	0.932
Range	0.33 –1.78	0.211–2.15	0.144–2.08
HbA_{1c}, %/mmol/mol			
Median	7.3/56	7.4/57	7.2/55
Range	5.3–12.3/34–111	4.7–9.0/28–75	5.5–11.2/35–99
Total daily insulin dose at baseline, units/kg*			
Median	0.339	0.315	0.306
Range	0–1.06	0–0.963	0–0.921

*Missing data: one patient missing ICA status (all four other autoantibodies positive); three patients missing baseline insulin dose.

any study drug (assigned to ATG/GCSF) was removed. No subjects required extended hospitalization or readmission due to cytokine release or serum sickness. In addition, no subjects who received ATG/GCSF or ATG alone developed a serious infection. There were no cases of grade 4 serum sickness or cytokine release. There were no grade 5 adverse events.

Among those having serum sickness, 12/20 (60.6%) and 17/21 (81.0%) had steroid treatment in the ATG/GCSF and the ATG only treatment groups, respectively. While the study was formally double-masked, adverse reactions may have effectively unmasked subjects assigned to active therapy. Presumptions regarding treatment assignment could have led to behaviors that contributed to the improved outcomes following ATG. The use of steroids was not significantly different between the two experimental treatment groups. There was no difference in AUC C-peptide at 1 year in subjects who used steroids to treat serum sickness versus those who were not exposed to steroids.

Adverse events labeled as musculoskeletal/connective tissue system were significantly higher in those who received GCSF. There were no significant differences in infection rate, neoplasm, or lymphatic cancers when comparing ATG/GCSF versus placebo or ATG only versus placebo. One patient in the ATG only group reported a thyroid papillary tumor ~1 month following the 2-year study end point, and one patient in the placebo group reported a benign ovarian cyst. Very few severe hypoglycemic events were reported. Only two patients in the placebo group reported a hypoglycemic event, and one patient in the ATG/GCSF group reported a hypoglycemic event.

CONCLUSIONS

In this phase IIb clinical trial, treatment of new-onset T1D (duration of diagnosis <100 days) with a single course of low-dose ATG (2.5 mg/kg) preserved C-peptide and reduced HbA_{1c} when compared with subjects treated with placebo 1 year after therapy. The combination of ATG (2.5 mg/kg) and pegylated GCSF (6 mg subcutaneously every 2 weeks for 6 doses) also lowered HbA_{1c} but was not statistically different from placebo with respect to AUC C-peptide.

In contrast to data from NOD mouse studies (12), low-dose ATG therapy but not low-dose ATG/GCSF preserved C-peptide in new-onset T1D. These results suggest that while the NOD model is a useful starting point, species differences (e.g., immune constituents, β-cell physiology) may limit the ability of this animal model to accurately predict effective treatments in human clinical trials. In addition, these data are in contrast to the findings in the randomized, single-masked pilot study in individuals with established T1D, which indicated that combination therapy with low-dose ATG and GCSF preserves C-peptide (15,16). The relatively small sample sizes of the pilot ATG and GCSF study as well as this phase IIb effort may have contributed to the disparate results.

Comparisons of low-dose ATG to previous efforts to utilize ATG products are

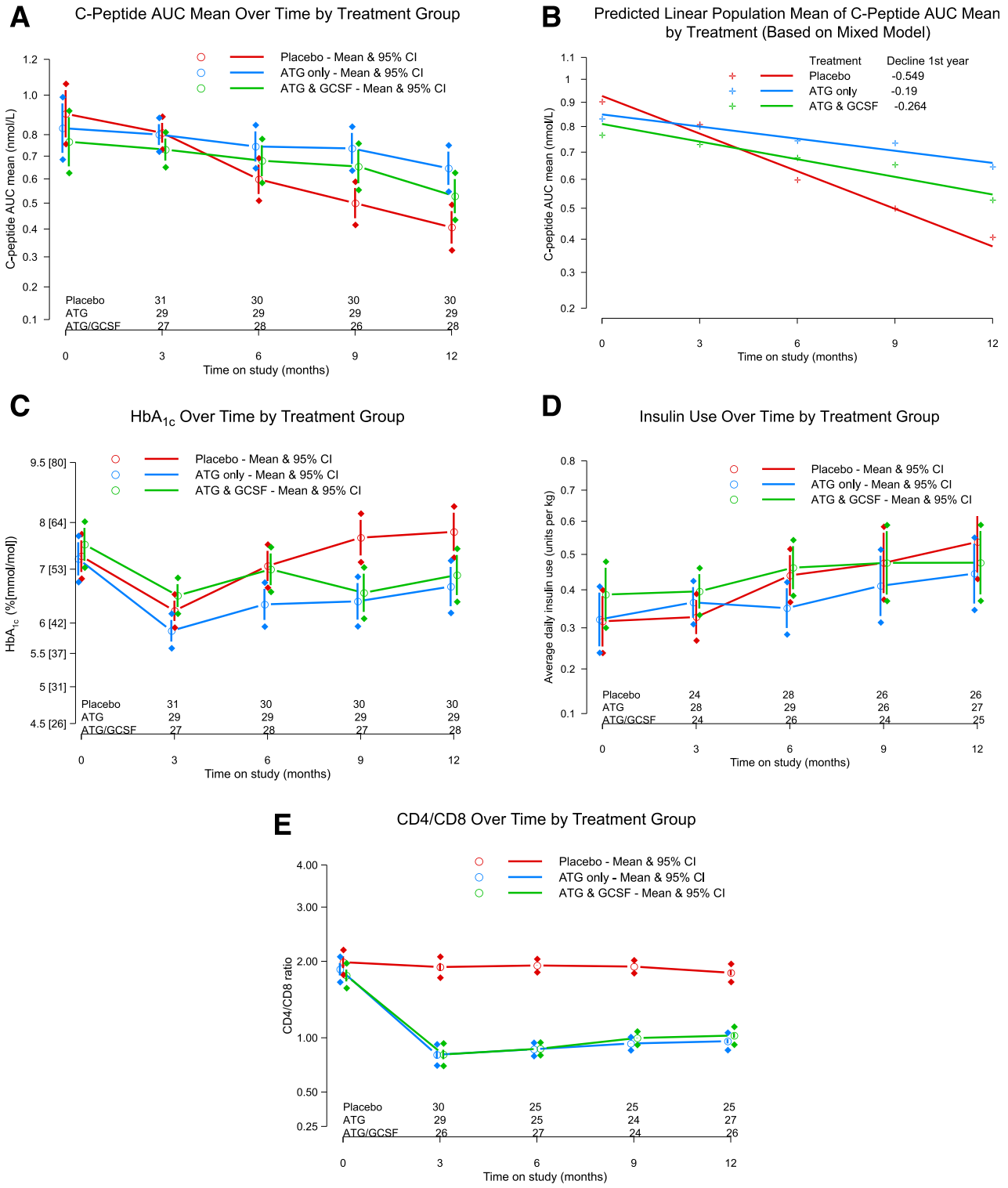

Figure 2—*A–E*: Effects of low-dose ATG and low-dose ATG/GCSF on C-peptide (*A*), mixed model predicted C-peptide (*B*), HbA_{1c} (*C*), insulin dose (*D*), and CD4/CD8 ratio (*E*). *A* and *C–E* show adjusted means and 95% CI at each time point. *B* shows the mixed model predicted population mean of the C-peptide AUC mean by treatment. In all figures, the placebo group is red, ATG/GCSF is green, and ATG alone is blue.

important. While different from the Sanofi ATG product (Thymoglobulin) used in this trial, Eisenbarth et al. (23) reported in 1985 that the combination of ATGAM and prednisone reduced HbA_{1c} and induced partial remission in two patients with new-onset T1D. Additional studies with ATGAM were abandoned as treatment-associated thrombocytopenia was thought to outweigh clinical benefit. In the Study of Thymoglobulin to Arrest Type 1 Diabetes (START), ATG monotherapy at a dose of 6.5 mg/kg failed to preserve C-peptide in new-onset T1D subjects aged 12–35 years (24). The robust cytokine release and serum sickness associated with higher-dose ATG may in part explain the difference in

Table 2—Adverse events

	ATG and GCSF		ATG only		Placebo	
Adverse effect category	Events	Patients	Events	Patients	Events	Patients
Skin and subcutaneous tissue disorder	6	5 (17.9)	10	7 (24.1)	7	4 (12.9)
All immune system disorders	33	21 (75.0)	38	23 (79.3)	0	0 (0)
Serum sickness only	20	20 (71.4)	21	21 (72.4)	0	0 (0)
Cytokine release syndrome only	11	10 (35.7)	17	14 (48.3)	0	0 (0)
Musculoskeletal and connective tissue	14	10 (35.7)	3	3 (10.3)	6	5 (16.1)
CD4 lymphocyte decrease or other*	42	21 (75.0)	43	22 (75.9)	4	3 (9.7)
General disorders and administration**	16	7 (25.0)	18	8 (27.6)	1	1 (3.2)
Endocrine disorders	1	1 (3.6)	1	1 (3.4)	7	3 (9.7)
Infections and infestations	14	9 (32.1)	9	7 (24.1)	16	9 (29.0)
Gastrointestinal disorders	7	5 (17.9)	5	3 (10.3)	6	6 (19.4)
Surgical and medical procedures	1	1 (3.6)	0	0 (0)	1	1 (3.2)
Psychiatric disorders	7	2 (7.1)	1	1 (3.4)	0	0 (0)
Injury, poisoning, and procedural complications	4	1 (3.6)	2	2 (6.9)	5	5 (16.1)
Nervous system disorders	4	4 (14.3)	11	4 (13.8)	5	2 (6.5)
Metabolism and nutrition disorders	7	4 (14.3)	4	2 (6.9)	4	4 (12.9)
Vascular disorders	0	0 (0)	2	2 (6.9)	1	1 (3.2)
Neoplasms: benign, malignant, and unspecified	0	0 (0)	1	1 (3.4)	1	1 (3.2)
Respiratory, thoracic, and mediastinal	2	2 (7.1)	3	2 (6.9)	1	1 (3.2)
Blood and lymphatic system disorder	1	1 (3.6)	1	1 (3.4)	2	2 (6.5)
Cardiac disorders	1	1 (3.6)	0	0 (0)	0	0 (0)
Ear and labyrinth disorders	1	1 (3.6)	0	0 (0)	0	0 (0)
Total	161	28 (100)	152	29 (100)	67	31 (100)

Data are *n* or *n* (%). *75% of the events were decreased lymphocytes. Others listed were decreased neutrophils, decreased white blood cells, increased alanine aminotransferase, increased alkaline phosphatase, and increased bilirubin. **Mostly fever and flu-like symptoms.

C-peptide preservation observed with low-dose ATG (14).

In this study, we observed the expected reduction in CD4 T cells following treatment with low-dose ATG but a relative preservation of CD8 T cells, yielding a low CD4/CD8 ratio. The pattern of CD4 T-cell reduction with CD8 T-cell preservation was observed in the pilot low-dose ATG/GCSF study (16), correlated with preservation of Treg, and was associated with clinical responses to anti-CD3 (25–27). In contrast, a 50% reduction in Treg with no change in CD4/CD8 ratio was observed in START (ATG, 6.5 mg/kg). These observations provide further support for the notion that low-dose ATG and low-dose ATG/GCSF

Table 3—Adverse events grades 3 and 4

	ATG and GCSF		ATG only		Placebo	
Adverse effect category	Events	Patients	Events	Patients	Events	Patients
All immune system disorders	14	12 (42.9)	15	15 (51.7)	0	0 (0)
Serum sickness only	12	12 (42.9)	15	15 (51.7)	0	0 (0)
Cytokine release syndrome only	2	2 (7.1)	0	0 (0)	0	0 (0)
CD4 lymphocyte decrease or other*	24	18 (64.3)	23	17 (58.6)	0	0 (0)
General disorders and administration**	4	2 (7.1)	1	1 (3.4)	1	1 (3.2)
Endocrine disorders	0	0 (0)	0	0 (0)	5	1 (3.2)
Infections and infestations	0	0 (0)	0	0 (0)	1	1 (3.2)
Gastrointestinal disorders	1	1 (3.6)	0	0 (0)	0	0 (0)
Surgical and medical procedures	0	0 (0)	0	0 (0)	1	1 (3.2)
Psychiatric disorders	3	1 (3.6)	0	0 (0)	0	0 (0)
Injury, poisoning, and procedural complications	2	1 (3.6)	1	1 (3.4)	1	1 (3.2)
Nervous system disorders	1	1 (3.6)	1	1 (3.4)	1	1 (3.2)
Metabolism and nutrition disorders	3	2 (7.1)	0	0 (0)	2	2 (6.5)
Neoplasms: benign, malignant, and unspecified	0	0 (0)	1	1 (3.4)	0	0 (0)
Blood and lymphatic system disorder	0	0 (0)	1	1 (3.4)	0	0 (0)
Total	52	28	43	29	12	31

Data are *n* or *n* (%). *Mostly decreased lymphocytes. Others listed were decreased neutrophils, decreased white blood cells, increased alanine aminotransferase, increased alkaline phosphatase, and increased bilirubin. **Mostly fever and flu-like symptoms.

are immunologically distinct from higher-dose ATG.

As we consider the potential application of low-dose ATG to future therapeutic approaches in T1D, we must consider its limitations. First, the addition of GCSF to low-dose ATG is associated with musculoskeletal side effects but fails to statistically preserve C-peptide. Given the costs, challenges of 12 weeks of therapy, and the side effects associated with GCSF, there seems little rationale, based on the 1-year data, to consider GCSF as a partner for ATG in subsequent combination approaches. Second, the use of low-dose ATG does not fully eliminate side effects associated with cytokine release or serum sickness. That said, the side-effect profile of low-dose ATG, while not inconsequential, is predictable and manageable. Third, given that the severity of serum sickness increases with repeated exposure, low-dose ATG is likely best suited as a classic "induction therapy" followed by "consolidation" and "maintenance" therapies when considering future interventions in T1D (28). Notably, there are ongoing efforts to develop humanized ATG products that might, if effective, markedly reduce if not eliminate serum sickness (29).

Low-dose ATG should be compared, both in terms of efficacy and safety, to other contemporary agents seeking to preserve C-peptide in T1D. While comparisons to all previous T1D interventions are beyond the scope of this article, we compare and contrast several recently studied immunotherapies. The monoclonal antibody anti-CD3 has been extensively studied in new-onset T1D (3,27,30,31). Initial studies included subjects within 6 weeks of diagnosis who received 14 daily intravenous infusions of anti-CD3 hOKT3γ1(Ala-Ala). At 1 year, anti-CD3 treatment provided for significant preservation of C-peptide (27). Fever, anemia, and pruritic urticarial rashes were common adverse events. Unfortunately, large international studies of anti-CD3 in new-onset T1D failed to demonstrate C-peptide preservation (30).

The Type 1 Diabetes TrialNet has also conducted trials testing anti–B-cell therapy with anti-CD20 (rituximab) and costimulation modification with CTLA4-Ig (abatacept) in new-onset T1D (32,33). Rituximab, administered intravenously in a four-dose course, slowed decline in C-peptide, reduced HbA_{1c}, and reduced insulin dose over a period of 1 year compared with placebo. Abatacept, given as 27 intravenous doses over 24 months, resulted in 59% higher adjusted C-peptide as compared with placebo at the primary outcome period of 2 years. To compare the relative experimental treatment effects across the rituximab, abatacept, and ATG/GCSF trials at 1 year, a model was fitted to the data of all three studies adjusting for baseline C-peptide, age, and sex. Notably, low-dose ATG treatment provided for a 57% increase in AUC C-peptide over placebo compared with 16% for rituximab and 23% for abatacept at 1 year. While the modeling accounts for differences across studies, it should be noted that the rate of C-peptide fall in the ATG study appears to be greater than that in the rituximab and abatacept trials.

Finally, the fusion protein LFA3-Ig (alefacept) was studied in new-onset T1D. Subjects received two 12-week courses of the study drug separated by a 12-week pause. While failing to meet its primary end point, a 2-h MMTT ($P = 0.065$), alefacept preserved C-peptide using a secondary end point, a 4-h MMTT (34). Importantly, 42.4% of subjects treated with alefacept experienced grade 3 or 4 adverse events. While alefacept remains worthy of ongoing study, its manufacturer's voluntary withdrawal of the drug from the market challenges its application as a primary target in future interventional studies. Collectively, the challenges of comparing and contrasting studies with unique patient populations, end points, and adverse event profiles speaks to the need for multiarm head-to-head comparisons of combination T1D immunotherapies.

In conclusion, this phase IIb study showed that one course of low-dose ATG (2.5 mg/kg) within 3 months of clinical diagnosis of T1D slowed decline of β-cell function and reduced HbA_{1c} for at least 1 year after therapy. Future studies should include continuous glucose monitoring to allow assessment of potential effects on glucose variability. Long-term follow-up of this study cohort will determine the duration of protection afforded by low-dose ATG. Low-dose ATG should be considered, either as a monotherapy or combination therapy, as a potential means for preventing T1D.

Funding. 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 the National Institutes of Health (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 through the cooperative agreements U01 DK061010, U01 DK061034, U01 DK061042, U01 DK061058, U01 DK085465, U01 DK085461, UC4 DK085466, U01 DK085476, U01 DK085499, U01 DK085509, U01 DK097835, U01 DK103266, U01 DK103282, U01 DK106984, U01 DK107014, and UC4 DK106993; the National Center for Research Resources through Clinical Translational Science Awards UL1TR001085, UL1TR001427, UL1TR001863, UL1TR001082, UL1TR000114, UL1TR001857, and UL1TR000445; JDRF; and the American Diabetes Association. Additional funding was provided by The Leona M. and Harry B. Helmsley Charitable Trust (2015PG-T1D032) and the McJunkin Family Charitable Foundation. Sanofi provided Thymoglobulin (ATG), Amgen provided Neulasta (GCSF) and placebo, and Roche Diabetes Care provided diabetes management supplies for the study.

Under the terms of the cooperative agreement funding mechanism used by the NIH, representatives from the sponsoring institutes of the NIH (National Institute of Diabetes and Digestive and Kidney Diseases) participated in the design and conduct of the study; interpretation of the data; preparation, review, and approval of the manuscript; and the decision to submit the manuscript for publication. The sponsor did not have the right or ability to veto submission for publication.

Duality of Interest. M.A.A. holds patent US8758761B2, Combination therapies for treating type 1 diabetes, which includes the use of ATG in combination with GCSF for the treatment of type 1 diabetes. Additional funding was provided by Sanofi. No other potential conflicts of interest relevant to this article were reported.

As per the clinical trials agreement between Amgen and NIH and separately between Sanofi and the University of Florida, Amgen and Sanofi were sent the draft manuscript prior to submission. Amgen and Sanofi did not comment or provide input on the manuscript. Sanofi and Amgen had no involvement with study management, data collection, data analysis, or manuscript preparation.

Author Contributions. M.J.H., D.A.S., J.S.S., and C.J.G. conceived of the study, obtained funding, collected data, and wrote the manuscript. J.P.K. obtained funding, performed the data analyses, and wrote the manuscript. B.N.B. performed the data analyses and wrote the manuscript. J.L.M., M.A.A., D.J.B., D.B., L.A.D., S.E.G., R.G., P.A.G., K.C.H., J.B.M., A.M., H.R., W.R., and D.M.W. collected data and edited the manuscript. M.J.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented at the 78th Scientific Sessions of the American Diabetes Association, Orlando, FL, 22–26 June 2018.

References

1. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet 2014;383:69–82

2. Silverstein J, Maclaren N, Riley W, Spillar R, Radjenovic D, Johnson S. Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. N Engl J Med 1988;319:599–604
3. Herold KC, Gitelman SE, Masharani U, et al. A single course of anti-CD3 monoclonal antibody hOKT3γ1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. Diabetes 2005;54:1763–1769
4. Ludvigsson J, Krisky D, Casas R, et al. GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. N Engl J Med 2012;366:433–442
5. Wherrett DK, Bundy B, Becker DJ, et al.; Type 1 Diabetes TrialNet GAD Study Group. Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial. Lancet 2011;378:319–327
6. Moran A, Bundy B, Becker DJ, et al.; Type 1 Diabetes TrialNet Canakinumab Study Group; AIDA Study Group. Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. Lancet 2013;381:1905–1915
7. Orban T, Bundy B, Becker DJ, et al.; Type 1 Diabetes TrialNet Abatacept Study Group. Costimulation modulation with abatacept in patients with recent-onset type 1 diabetes: follow-up 1 year after cessation of treatment. Diabetes Care 2014;37:1069–1075
8. Pescovitz MD, Greenbaum CJ, Bundy B, et al.; Type 1 Diabetes TrialNet Anti-CD20 Study Group. B-lymphocyte depletion with rituximab and β-cell function: two-year results. Diabetes Care 2014;37:453–459
9. Schatz D, Gale EAM, Atkinson MA. Why can't we prevent type 1 diabetes? Maybe it's time to try a different combination. Diabetes Care 2003;26:3326–3328
10. Haller MJ, Gottlieb PA, Schatz DA. Type 1 diabetes intervention trials 2007: where are we and where are we going? Curr Opin Endocrinol Diabetes Obes 2007;14:283–287
11. Ludvigsson J. Combination therapy for preservation of beta cell function in type 1 diabetes: new attitudes and strategies are needed! Immunol Lett 2014;159:30–35
12. Parker MJ, Xue S, Alexander JJ, et al. Immune depletion with cellular mobilization imparts immunoregulation and reverses autoimmune diabetes in nonobese diabetic mice. Diabetes 2009;58:2277–2284
13. Haller MJ, Atkinson MA, Wasserfall CH, et al. Mobilization without immune depletion fails to restore immunological tolerance or preserve beta cell function in recent onset type 1 diabetes. Clin Exp Immunol. 2016;183:350–357
14. Gitelman SE, Gottlieb PA, Rigby MR, et al.; START Study Team. Antithymocyte globulin treatment for patients with recent-onset type 1 diabetes: 12-month results of a randomised, placebo-controlled, phase 2 trial. Lancet Diabetes Endocrinol 2013;1:306–316
15. Haller MJ, Gitelman SE, Gottlieb PA, et al. Anti-thymocyte globulin/G-CSF treatment preserves β cell function in patients with established type 1 diabetes. J Clin Invest 2015;125:448–455
16. Haller MJ, Gitelman SE, Gottlieb PA, et al. Antithymocyte globulin plus G-CSF combination therapy leads to sustained immunomodulatory and metabolic effects in a subset of responders with established type 1 diabetes. Diabetes 2016;65:3765–3775
17. Couri CE, Oliveira MC, Stracieri AB, et al. C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA 2009;301:1573–1579
18. American Diabetes Association. Glycemic targets. Sec. 6. In *Standards of Medical Care in Diabetes—2017*. Diabetes Care 2017;40(Suppl. 1): S48–S56
19. Bundy BN, Krischer JP; Type 1 Diabetes TrialNet Study Group. A model-based approach to sample size estimation in recent onset type 1 diabetes. Diabetes Metab Res Rev 2016;32: 827–834
20. Simon R, Wittes RE, Ellenberg SS. Randomized phase II clinical trials. Cancer Treat Rep 1985; 69:1375–1381
21. National Cancer Institute. *Common Terminology Criteria for Adverse Events (CTCAE)*. Version 4.0. U.S. Department of Health and Human Services, May 2009 (NIH publ. no. 09-5410)
22. Agresti A. *Categorical Data Analysis*. New York, NY, John Wiley & Sons, 1990
23. Eisenbarth GS, Srikanta S, Jackson R, et al. Anti-thymocyte globulin and prednisone immunotherapy of recent onset type 1 diabetes mellitus. Diabetes Res 1985;2:271–276
24. Gitelman SE, Gottlieb PA, Felner EI, et al.; ITN START Study Team. Antithymocyte globulin therapy for patients with recent-onset type 1 diabetes: 2 year results of a randomised trial. Diabetologia 2016;59:1153–1161
25. Tooley JE, Vudattu N, Choi J, et al. Changes in T-cell subsets identify responders to FcR-nonbinding anti-CD3 mAb (teplizumab) in patients with type 1 diabetes. Eur J Immunol 2016;46: 230–241
26. Long SA, Thorpe J, DeBerg HA, et al. Partial exhaustion of CD8 T cells and clinical response to teplizumab in new-onset type 1 diabetes. Sci Immunol 2016;1:eaai7793
27. Herold KC, Hagopian W, Auger JA, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med 2002;346: 1692–1698
28. Skyler JS. Immune therapy for treating type 1 diabetes: challenging existing paradigms. J Clin Invest 2015;125:94–96
29. Kuroiwa Y, Kasinathan P, Sathiyaseelan T, et al. Antigen-specific human polyclonal antibodies from hyperimmunized cattle. Nat Biotechnol 2009;27:173–181
30. Sherry N, Hagopian W, Ludvigsson J, et al.; Protégé Trial Investigators. Teplizumab for treatment of type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial. Lancet 2011;378:487–497
31. Aronson R, Gottlieb PA, Christiansen JS, et al.; DEFEND Investigator Group. Low-dose otelixizumab anti-CD3 monoclonal antibody DEFEND-1 study: results of the randomized phase III study in recent-onset human type 1 diabetes. Diabetes Care 2014;37:2746–2754
32. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, et al.; Type 1 Diabetes TrialNet Anti-CD20 Study Group. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. N Engl J Med 2009;361:2143–2152
33. Orban T, Bundy B, Becker DJ, et al.; Type 1 Diabetes TrialNet Abatacept Study Group. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised double-masked controlled trial. Lancet 2011;378:412–419
34. Rigby MR, DiMeglio LA, Rendell MS, et al.; T1DAL Study Team. Targeting effector memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomized, double-blind, placeo-controlled phase 2 trial. Lancet Diabetes Endocrinol 2013;1:284–294