

CORRESPONDENCE



Intralymphatic Injection of Autoantigen in Type 1 Diabetes

TO THE EDITOR: Residual insulin secretion decreases complications and improves quality of life in patients with type 1 diabetes. However, effective interventions to preserve residual beta-cell function are lacking. Antigen-based therapy requires adequate presentation to T cells. Treatment with antigen-based therapy with the use of glutamic acid decarboxylase (GAD65) has been encouraging but not sufficiently effective.¹

To render the presentation of GAD65 antigen to T cells in the lymph nodes more efficient than has previously been described,^{2,3} we now report the administration of GAD65 autoantigen directly into an inguinal lymph node rather than subcutaneously. We also added oral vitamin D therapy as a potential immune modulator, although one trial showed that beta-cell function was not preserved in patients who received vitamin D alone.⁴

DIAGNODE-1 (GAD-Alum [Diamyd] Administered into Lymph Nodes in Combination with Vitamin D in Type 1 Diabetes; ClinicalTrials.gov number, NCT02352974), a pilot open-label clinical trial, involved six adult patients who were 20 to 22 years of age and had had incident diabetes for less than 6 months. All the patients were GAD65 antibody-positive and had fasting C-peptide levels greater than 0.12 nmol per liter (0.36 ng per milliliter). They received an injection of 4 μ g of alum-formulated GAD65 (GAD-alum) into an inguinal lymph gland under direct ultrasonographic guidance, followed by two intranodal booster injections at 1-month intervals. Each patient also received vitamin D (calciferol) in an oral solution (2000 U per day) over 4 months, starting 1 month before the first GAD-alum injection. All patients provided written informed consent. The trial was approved by the research ethics committee at Linköping University, Linköping, Sweden, and by the Medical Products Agency, Uppsala, Sweden.

Beta-cell function was estimated with mixed-meal tolerance tests. Immune function was assessed by means of cell-proliferation assays, flow cytometry, and measurement of cytokine levels through advanced techniques (Bio-Rad and Lumines) and GAD65 autoantibodies, including subclasses.

There were no apparent treatment-related adverse events, except for mild transient injection-site reactions. The fasting and maximum stimulated C-peptide levels from baseline to 6 months did not decrease in six patients. After 15 months, the mean area under the curve of the serum C-peptide level remained stable in four patients, with an increase of 34% in the fasting C-peptide level (Fig. 1A). The glycated hemoglobin level (Fig. 1B) and insulin dose (Fig. 1C) decreased in each patient. Immunologic markers showed type 2 helper T-cell (Th2) up-regulation, with a stepwise increase of Th2 markers (e.g., interleukin-13 and interleukin-5) after each GAD-alum injection and a decrease of type 1 helper T cell (Th1) markers (e.g., interferon- γ and tumor necrosis factor α) along with signs of T-cell up-regulation (e.g., an increase in interleukin-2 and interleukin-10 levels) after 15 months.

Direct injection of GAD-alum into the lymph

THIS WEEK'S LETTERS

- 697 Intralymphatic Injection of Autoantigen in Type 1 Diabetes
- 699 Antiretroviral Therapy for Perinatal HIV Prevention
- 700 Trial of ZMapp for Ebola Virus Infection
- 701 Von Willebrand's Disease

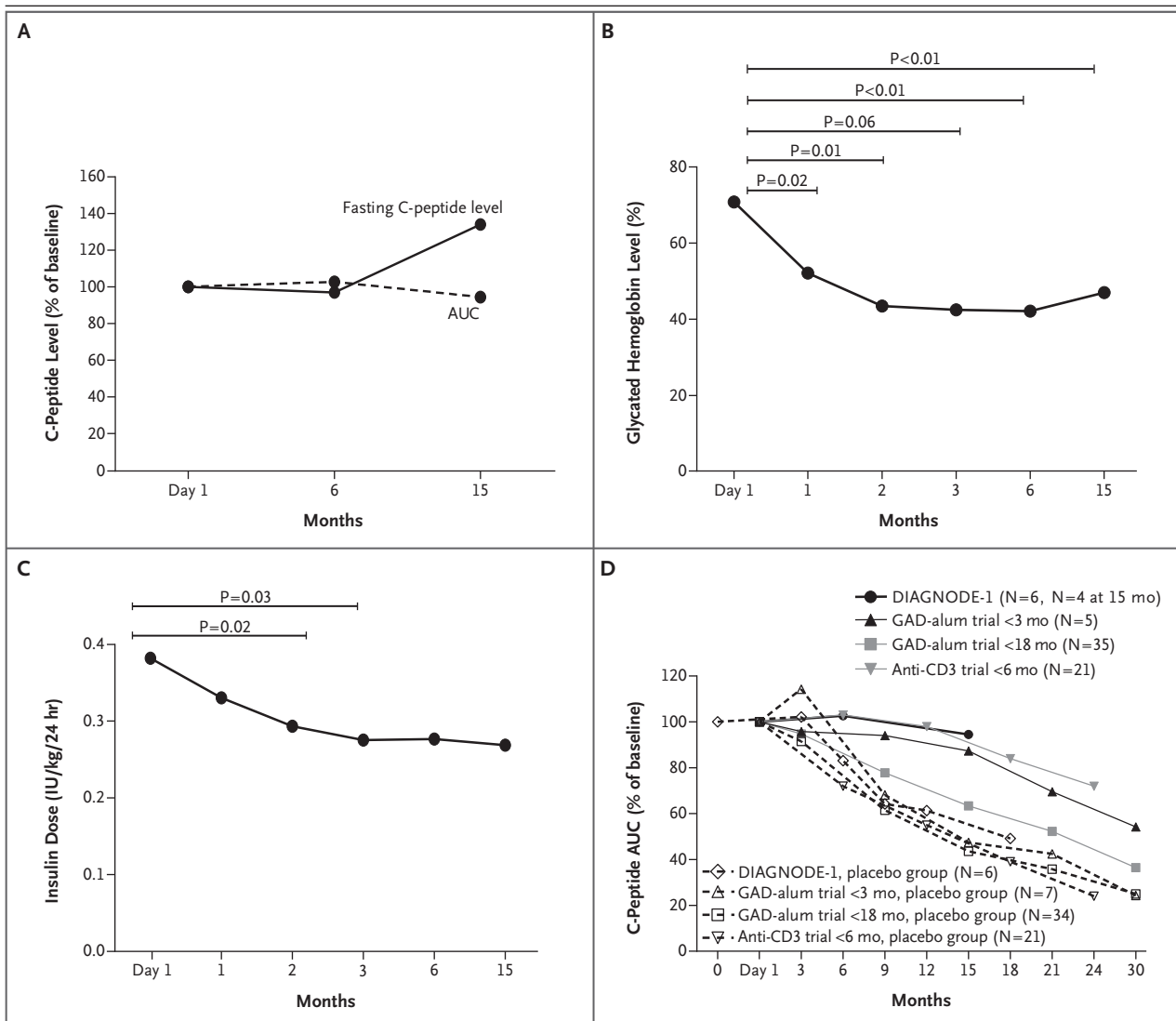


Figure 1. Changes in C-Peptide and Glycated Hemoglobin Levels and Insulin Doses over Time.

Panel A shows fasting C-peptide levels and the mean area under the curve (AUC) of the serum C-peptide level in the six patients after a mixed-meal tolerance test. Panel B shows that the glycated hemoglobin level decreased with time. Panel C shows that the insulin requirement decreased with time. Panel D shows the normalized C-peptide AUC in patients in the DIAGNODE-1 trial as compared with some similar populations of patients who had received placebo or active immune intervention with subcutaneous GAD-alum (alum-formulated glutamic acid decarboxylase) or anti-CD3 monoclonal antibodies.

node with administration of oral vitamin D was associated with preservation of residual beta-cell function in six patients with type 1 diabetes, as compared with historical age-matched patients with type 1 diabetes who received vitamin D alone⁴ or, in other immune-intervention trials,^{3,5} placebo. Our results appear to be similar to promising results observed in patients who received other immune interventions with or without placebo⁵ (Fig. 1D).

Johnny Ludvigsson, M.D., Ph.D.
Jeanette Wahlberg, M.D., Ph.D.
Rosaura Casas, Ph.D.

Linköping University
Linköping Sweden
johnny.ludvigsson@liu.se

Supported by grants from Barndiabetesfonden (the Swedish Child Diabetes Foundation), Diabetesfonden (the Swedish Diabetes Foundation), Forskningsrådet i Sydöstra Sverige (the Research Council of Southeast Sweden), and an unrestricted grant from Diamyd Medical.

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

1. Beam CA, MacCallum C, Herold KC, Wherrett DK, Palmer J, Ludvigsson J. GAD vaccine reduces insulin loss in recently diagnosed type 1 diabetes: findings from a Bayesian meta-analysis. *Diabetologia* 2017;60:43-9.
2. Ludvigsson J, Krisky D, Casas R, et al. GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. *N Engl J Med* 2012;366:433-42.
3. Wherrett DK, Bundy B, Becker DJ, et al. Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients

with recent-onset type 1 diabetes: a randomised double-blind trial. *Lancet* 2011;378:319-27.

4. Bizzarri C, Pitocco D, Napoli N, et al. No protective effect of calcitriol on beta-cell function in recent-onset type 1 diabetes: the IMDIAB XIII trial. *Diabetes Care* 2010;33:1962-3.
5. Herold KC, Gitelman SE, Masharani U, et al. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(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;54:1763-9.

DOI: 10.1056/NEJMc1616343

Antiretroviral Therapy for Perinatal HIV Prevention

TO THE EDITOR: In reporting on the results of the Promoting Maternal and Infant Survival Everywhere (PROMISE) trial, Fowler et al. (Nov. 3 issue)¹ highlight the various risks and benefits associated with three different antiretroviral therapy (ART) regimens in African infants with perinatal exposure to the human immunodeficiency virus (HIV). However, given the continued exposure to ART in African children in the age of “Option B+” (a program in which all pregnant and breast-feeding women with HIV-1 infection begin lifelong ART, regardless of their CD4+ cell counts), it is necessary for these trials to continue beyond the early postpartum period into early childhood. In 2014, there were approximately 1.2 million pregnant women with HIV infection in southern and eastern Africa.²

Studies have shown cardiac risks associated with in utero exposure to zidovudine³ and poor growth outcomes at 5 years of age with exposure to nevirapine or zidovudine.⁴ However, few studies focus on longer-term outcomes in sub-Saharan African populations, and most involve infants in the first few weeks of life or infants younger than 6 weeks of age. This needs to be changed because of the large number of children who will have prolonged exposure to ART in utero and through breast-feeding with Option B+.

Janet M. Wojcicki, Ph.D., M.P.H.

University of California, San Francisco
San Francisco, CA
wojcickij@peds.ucsf.edu

No potential conflict of interest relevant to this letter was reported.

1. Fowler MG, Qin M, Fiscus SA, et al. Benefits and risks of antiretroviral therapy for perinatal HIV prevention. *N Engl J Med* 2016;375:1726-37.
2. 2015 Progress report on the global plan. Geneva: UNAIDS,

2015 (http://www.unaids.org/sites/default/files/media_asset/JC2774_2015ProgressReport_GlobalPlan_en.pdf).

3. Sibiude J, Le Chenadec J, Bonnet D, et al. In utero exposure to zidovudine and heart anomalies in the ANRS French perinatal cohort and the nested PRIMEVA randomized trial. *Clin Infect Dis* 2015;61:270-80.
4. Owor M, Mwatha A, Donnell D, et al. Long-term follow-up of children in the HIVNET 012 perinatal HIV prevention trial: five-year growth and survival. *J Acquir Immune Defic Syndr* 2013;64:464-71.

DOI: 10.1056/NEJMc1616287

THE AUTHORS REPLY: As Wojcicki notes, although most new HIV infections in infants can now be prevented through the use of ART in pregnant and breast-feeding women, the longer-term effects of fetal exposure to HIV and multiple forms of ART in uninfected infants who are exposed to HIV have, unfortunately, remained an unexplored frontier of research.¹ Each year, an estimated 1.5 million women with HIV infection give birth,^{2,3} and with the rollout of universal treatment guidelines,⁴ most of their infants will have prolonged exposure to ART through fetal exposure and breast milk.

The PROMISE trial is following children through 24 months of age for growth and safety assessments, and a separate study (Developmental and Growth Outcomes for ARV Exposed HIV Uninfected African Children) involving children through 5 years of age at two sites is under way to compare the neurodevelopment of uninfected children in our trial with that of children who have not been exposed to HIV. In addition, the PROMISE investigators have received funding from the U.S. President's Emergency Plan for AIDS Relief for a 5-year follow-up study involving a safety cohort of approximately 2000 mothers and children at PROMISE sites in Africa that had high enrollments in the PROMISE trial. This