Etanercept Treatment in Children with New Onset Type 1 Diabetes: Pilot Randomized, Placebo-Controlled, Double Blind Study

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Objective: To gather preliminary data on the feasibility and efficacy of etanercept therapy to prolong endogenous insulin production in newly diagnosed pediatric patients with type 1 diabetes mellitus.

Research design and Methods: A 24-week double-blind, randomized, placebo-controlled study conducted at the Diabetes Center, Women and Children’s Hospital of Buffalo. Eighteen subjects (11M/7F, age 7.8-18.2 years) were randomized to receive either placebo (P) or etanercept (E). Inclusion criteria included age 3-18 years, GAD-65 and/or ICA positivity, HbA1c above 6%, 3 insulin injections per day, WBC 3,000-10,000, platelets >100,000, and normal liver and renal function. Intention to treat analysis was used.

Results: HbA1c at week 24 was lower in the etanercept (5.91 ± 0.5%) compared to placebo group (6.98 ± 1.2%; p<0.05) with a higher percent decrease from baseline compared to the placebo (E 0.41 ± 0.1 vs. P 0.18 ± 0.21; p<0.01). The percent change in c-peptide AUC from baseline to week 24 showed a 39% increase in the etanercept group and a 20% decrease in the placebo group (p<0.05). From baseline to week 24 insulin dose decreased 18% in the etanercept group compared to 23% increase in the placebo group (p<0.05). Seventeen patients completed, none withdrew because of adverse events.

Conclusions: In this small pilot study, treatment of pediatric patients newly diagnosed with type 1 diabetes mellitus with etanercept resulted in lower HbA1c, increased endogenous insulin production, suggesting preservation of beta cell function. A larger study is needed to further explore safety and efficacy.
Type 1 diabetes mellitus is a T-cell mediated autoimmune disease characterized by selective destruction of insulin-producing β-cells within the pancreatic islet. This chronic disease affects 1:400-600 youth and poses significant medical and psychological burden on patients and their families (1-3). In most patients, diagnosis and treatment is followed by a “partial remission period” (honeymoon period) during which transient partial recovery of endogenous insulin production occurs. Eventually, there is progressive, irreversible β-cell demise leaving the patient totally dependent on exogenous insulin administered via multiple daily injections or continuous subcutaneous insulin infusion. Thus, the partial remission period represents a window of opportunity to halt the progression of the disease. Several clinical studies are testing agents which may alter the natural history of type 1 diabetes mellitus.

Tumor Necrosis Factor-alpha (TNFα) and other cytokines play a role in the autoimmune process leading to pancreatic destruction (4; 5). Evidence suggesting that TNFα plays an active role in the pathogenesis of type 1 diabetes mellitus is derived from in vitro studies and animal models. In the non-obese diabetic (NOD) mouse, TNFα mRNA is produced by CD4+ T cells within inflamed islets during the development of diabetes (6). In vitro models show that TNFα potentiates the direct functional inactivation and destruction of β-cells by other cytokines such as interleukin-1β and interferon-γ (7-11). Transgenic mice with increased β-cell expression of TNFα have significant lymphocytic insulinitis, which is abrogated in TNF receptor null mice (12). These findings support the role of TNFα in signaling lymphocytic invasion, promoting local inflammation within pancreatic islets, and contributing to cytokine-induced β-cell destruction.

Etanercept is a recombinant soluble TNFα receptor fusion protein that binds to TNFα. It acts by clearing TNFα from the circulation, thereby blocking the biological activity of this inflammatory cytokine. Although etanercept is used in the treatment of many autoimmune diseases including ankylosing spondilitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis and rheumatoid arthritis (RA), it has never been tested in youth with type 1 diabetes mellitus (13; 14). We hypothesized that the administration of etanercept to children newly diagnosed with type 1 diabetes mellitus may prolong the partial remission period. The aim of this pilot study was to gather preliminary data on the feasibility and efficacy of etanercept administration to pediatric patients recently diagnosed with type 1 diabetes mellitus. The primary end points of this study were percent change from baseline for HbA1c and for c-peptide area under the curve (AUC). Secondary end points were insulin dose change from baseline and number of insulin injections discontinued, if any.

METHODS
This was a randomized, double-blind, placebo-controlled feasibility study lasting 24 weeks, followed by a 12-week wash-out period. Inclusion criteria were as follows: male and female subjects with type 1 diabetes mellitus aged 3-18 years, positive GAD-65 and/or islet cell antibody, HbA1c at diagnosis above 6%, insulin regimen with 3 injections daily, white blood count between 3,000-10,000 and platelets >100,000, normal ALT and AST, creatinine <1.8 mg/dl, and type 1 diabetes
mellitus duration equal or less than 4 weeks. However, in order to accommodate parental schedules, this window was lengthened resulting in a 1.7-5.7 week interval between diabetes diagnosis and baseline. Exclusion criteria were as follows: intravenous antibiotics at diagnosis or within 14 days of study entry, body mass index over 85th percentile for age and gender, unstable household unable to comply with study protocols, evidence of psychiatric disease in the study subject and/or primary care taker, and chronic disease, including additional autoimmune disorders with the exception of euthyroid autoimmune thyroiditis. The study was approved by the institutional review boards of the Women and Children’s Hospital of Buffalo and the University at Buffalo and was conducted in accordance with the Declaration of Helsinki. Subjects and their parents signed informed consent and assent. The study was registered at www.clinicaltrial.gov (NCT00730392).

All patients received a 3-injection insulin regimen with Humalog and NPH pre-breakfast, Humalog pre-dinner and NPH at bed time. Patients were instructed to measure blood glucose (BG) by finger stick before breakfast, lunch, dinner, bed time, 2:00 am as needed, and when having symptoms of hypoglycemia. Insulin adjustments were made throughout the study at clinic visits and/or by telephone/email in between study visits.

Study visits occurred every 4 weeks during the study drug period and 4 and 12 weeks following discontinuation of study drug. Randomization was stratified based on HbA1c level of 9.5% at diagnosis. Study subjects were randomized in a 1:1 ratio to subcutaneous injections of placebo or etanercept. The study drug, provided by Immunex/Amgen, was administered at a dose of 0.4 mg/Kg up to maximum dose of 25 mg/dose subcutaneously twice weekly (Monday/Thursday, Tuesday/Friday or Wednesday/Saturday). The study drug was stored in the pharmacy of the Women and Children’s Hospital of Buffalo and reconstituted with 1 milliliter of bacteriostatic water by the pharmacist according to the master randomization table provided by Immunex/Amgen. Every 4 weeks parents were provided with pre-filled and refrigerated syringes of study drug and returned empty syringes.

Study procedure at every visit included: laboratory work (cell blood counts, biochemical profile inclusive of liver and kidney function tests, and HbA1c), clinic visit (physical exam, BG review and insulin adjustment), and study drug dispensation. Menstruating females also had urine hCG performed. Antinuclear antibody titer was measured at baseline, weeks 12 and 24 of treatment, and week 12 of wash-out. At baseline and week 24, a Boost Meal Test was performed (6 ml/Kg up to a maximum of 360 ml). C-peptide was measured at 0, 30, 60, 120 minutes and area under the curve (AUC) was calculated using the trapezoidal rule (15). The cell blood count and differential, biochemical profiles, HbA1c, and Antinuclear Antibody (ANA) titer were performed in the laboratory of the Women and Children’s Hospital of Buffalo (Kaleida Health). The HbA1c was measured by high-performance liquid chromatography with Bio-Rad variant (Bio-Rad, Richmond, CA). C-peptide was measured by immunoassay; ICA and GAD-65 antibodies by radiobinding at Nichols Institute laboratory (San Juan Capistrano, CA).
Preliminary data were not available to perform power calculations. Historically, interventions which result in a change in HbA1c of 1% (SD \( \leq 1.0 \)) are accepted as clinically relevant. A priori power analysis indicated that a total sample size of 30 subjects (15 control, 15 treatment) would have 86% power to detect a 1% difference in HbA1c level (SD = 1.0) after 24 weeks of treatment (\( \alpha = 0.05; \) effect size = 1.1). Due to the slow recruitment the principal investigator (PI) decided to halt the study before reaching the planned number of participants. The PI felt that should these preliminary results be favorable, they could be used as basis for a larger efficacy and safety study to be proposed to TrialNet.

The data are expressed as mean ± SD with the exception of Figure 2 where values are expressed as mean ± SEM. Comparisons between groups at each time point were performed using the Wilcoxon Mann Whitney exact method test. This method was used because of the small sample size and the fact that it does not rely on large sample size approximation. Note that the linear model based method was not used to handle repeated measures as this approach is reliable only for the large samples. The Kendall’s tau b coefficient was used to test for associations between numerical outcomes. All statistical analyses were conducted using Statistical Analysis System (SAS version 9.1) Intention to treat analysis was performed.

RESULTS

Nineteen patients and their parents signed informed consent/assent. Intention to treat analysis was performed for the data of the 18 randomized patients (Fig. 1). The characteristics of study participants at baseline were not statistically different between groups (Table 1) Study protocol IRB and FDA procedures were in place in June 2002; the study took place between October 2002 and October 2007. Reasons for slow recruitment were: 1) parental concern over potential side effects, and 2) lack of “stable household environment” which was necessary to ensure adherence with the study protocol and overall safety for the patient receiving the study drug.

Efficacy Data: Figure 2 shows mean HbA1c values and percent decrease throughout the study. HbA1c values were higher in the etanercept compared to placebo group at both baseline and week 4 of treatment (difference not statistically significant). From week 8, the HbA1c values were lower in the etanercept group, with statistical significance at week 24 in the etanercept (5.91 ± 0.5%) compared to placebo (6.98 ± 1.2%; \( p<0.05; \) Fig. 2A). Moreover, from week 8 through week 24 of treatment, the percent decrease in HbA1c from baseline was consistently higher in favor of the etanercept group (Fig. 2B). The HbA1c values continued to be lower in the etanercept compared to placebo at the 4- and 12-week wash-out observations, but this reached statistical significance only at the +4-week wash out visit ( \( p<0.05; \) Fig. 2A). However, the percent HbA1c decrease from baseline continued to be statistically greater in the etanercept compared to placebo at both week +4 and week +12 of wash-out (Fig. 2B).

Blood glucose levels pre-breakfast, pre-dinner and pre-bed times for a 7-day period preceding baseline and week 24 were similar at baseline between the groups. At week 24, they demonstrated a trend towards lower blood sugar averages fasting (111±27 vs. 158±69 mg/dl; \( p=0.18 \)), pre-dinner (149±44
vs.208 ±55 mg/dl, p=0.054) and pre-bedtime (129±24 vs.198±46; p<0.01) in the etanercept and placebo group, respectively.

C-peptide AUC (ng/ml/hour) in the etanercept group increased from 3.1 ± 1.2 at baseline to 3.9 ± 1.6 at week 24 while in the placebo group it decreased from 4.7 ± 2.2 at baseline to 3.6 ± 2.0 at week 24. These changes resulted in a mean relative percent increase in c-peptide AUC from baseline to week 24 of 39% in the etanercept group while the placebo group exhibited a 20% decrease compared to baseline (p<0.05). In the etanercept group, all subjects but one experienced stable or increased c-peptide AUC, while in the placebo group, all subjects but one showed a decline in c-peptide AUC (Fig. 3). There was no association between type 1 diabetes mellitus duration and c-peptide or HbA1c at either baseline or week 24.

The number of insulin injections in the etanercept and placebo groups did not differ appreciably. However, the insulin dose change between baseline and week 24 showed a mean decrease of 18% in the etanercept compared to a mean increase of 23% increase in the placebo group (p<0.05). There was no association between relative difference in c-peptide and insulin dose from baseline to week 24.

**Safety Data:** There were no severe adverse events in either group. The frequency of events was similar in the two groups with the following exceptions: 3 very mild episodes of self-resolving paresthesia in one subject in the etanercept and none in the placebo group; cold symptoms, reported twice as frequently in the etanercept compared to the placebo group; and abdominal pain with 6/9 episodes reported by one patient receiving etanercept. None of these events caused significant disruption of daily activities or significant absence from school.

Cell Blood Count (CBC) with differential and biochemical profile (BCP), including liver and renal function tests were unremarkable in the two groups. The majority of CBC or BCP abnormalities was present at baseline, non-clinically significant, and did not worsen during the study.

Positive ANA titer was detected at baseline in 5 subjects assigned to etanercept and 2 assigned to placebo. One of the ANA results at baseline in the etanercept group is missing. This subject had positive ANA in the samples drawn thereafter. None of the subjects with negative results at baseline converted to positive ANA status in the etanercept group and one subject converted to positive in the placebo group after discontinuation of study drug.

**CONCLUSIONS**

This is the first placebo-controlled pilot study to gather data on the feasibility of etanercept to prolong endogenous insulin production in youth with new onset type 1 diabetes mellitus. A 24-week course of etanercept administered to newly diagnosed pediatric patients with type 1 diabetes mellitus resulted in lower HbA1c in the etanercept compared to the placebo group at the end of the treatment period, with a significantly different HbA1c percent change from baseline favoring the etanercept arm. This finding was associated with a mean increase from baseline of 39% increase in c-peptide AUC in response to a meal test in the etanercept group as opposed to a mean decrease of 20% observed in the placebo group, which is similar to declines published in the literature (16). Thus, both primary end points of this trial were
met. In addition, glycemic levels during a 7-day period preceding the week-24 visit showed a lower trend for the etanercept group, a finding consistent with the HbA1c results.

When the total daily insulin dose at week 24 was compared to baseline, a significant difference was found with a mean decrease of 18% in the etanercept group compared to a 23% increase in insulin usage in the placebo group. This difference in insulin usage was paralleled by an increase in c-peptide AUC during meal test in the etanercept treated group compared to a decline in the placebo group, as noted above. These results suggest that the decrease in exogenous insulin usage is due to preservation of endogenous insulin production. The improved metabolic control experienced by the etanercept group is important as patients and health care providers strive to achieve near-normal control as it correlates with improved clinical outcomes (17).

Serum levels of TNFα are increased in patients newly diagnosed with type 1 diabetes mellitus compared to controls (18). Even after β-cell function is completely lost, serum TNFα levels remain elevated in type 1 diabetes mellitus (19) suggesting that the hyperglycemic state is associated with chronic inflammation. Anti-TNFα agents, including etanercept, have an established role in the treatment of RA and other autoimmune conditions. In an adult patient with type 1 diabetes mellitus and RA, following initiation of adalimumab (monoclonal antibody against TNFα), fructosamine levels decreased without any reduction in ESR and CRP or in insulin usage (20). In another report describing three patients with insulin resistance as measured by HOMA-index, chronic treatment with infliximab for either RA or psoriatic arthritis resulted in significant improvement in insulin sensitivity. One patient with type 2 diabetes reverted to a diagnosis of impaired glucose tolerance and stopped insulin therapy. The authors hypothesize that TNFα blockade resulted in improved insulin signaling (21).

Our findings suggest that etanercept administration at doses customarily used for the treatment of RA is well-tolerated in pediatric patients with new onset type 1 diabetes mellitus. In addition, our data show preservation of β-cell function with improvement in glycemic control following a 24-week course of etanercept therapy. While these results are promising, it should be recognized that patients receiving etanercept or other anti-TNF agents must be carefully monitored for other autoimmune conditions and infections, including tuberculosis, which occurred in about 0.007% of etanercept treated patients in trials enrolling more than 15,000 patients in the United States and Canada. In fact, many of the agents currently being tested to prolong the remission period in type 1 diabetes mellitus are likely to have significant effects on the immune system. Thus, the goal of halting pancreatic islet β-cell demise must be weighed against possible complications from agents that might achieve this goal. In addition, the potential need for continuous therapy is an important factor weighing in the cost-benefit balance, as compared to other potential immunotherapies. For example, a single 14-day course of anti-CD3 monoclonal antibody resulted in preserved β-cell function for at least two years following onset of diabetes in 67% of subjects assigned to intervention compared to 26% of subjects receiving placebo. However, etanercept therapy...
does not require hospitalization and side effects were minimal compared to those observed during anti-CD3 monoclonal antibody infusion (16).

A larger study is needed to confirm these preliminary data that show that etanercept may modulate the progression of type 1 diabetes mellitus and to further address the risk/benefit ratio of etanercept therapy in this population. Given the complex pathogenesis of type 1 diabetes mellitus, the use of etanercept in new onset type 1 diabetes mellitus may need to be coupled with another agent modulating T cells. Lastly, the possibility exists that the use of etanercept may be explored to examine if treatment with etanercept could prevent or delay diabetes development in those individuals at high risk for the disease.

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The Principal Investigator of this study, Dr. Teresa Quattrin, had full access to the data in the study and takes responsibility for the integrity and the accuracy of data analysis. Data analysis was conducted by Dr. Jihnhee Yu, an independent statistician in the Department of Biostatistics, State University of New York at Buffalo. The results of this independent statistical analysis are reported in the manuscript.

The Principal Investigator of this study, Dr Teresa Quattrin, and all the co-authors have no conflict of interest, including specific financial interests, relationship and affiliation relevant to the subject matter or material discussed in the manuscript.

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REFERENCES
responses and clinical parameters for at least 2 years after onset of type 1 diabetes. Diabetes 54:1763-1769, 2005
Table 1 - Characteristics of Study Participants at Diagnosis

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<thead>
<tr>
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<th>Etanercept</th>
<th>Placebo</th>
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<tr>
<td>Gender (M/F)</td>
<td>8/2</td>
<td>3/5</td>
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<tr>
<td>Age (yrs)</td>
<td>12.5 ± 3.3</td>
<td>12.4 ± 3.6</td>
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<td>Height (cm)</td>
<td>154.4 ± 18.3</td>
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<tr>
<td>Weight (kg)</td>
<td>51.1 ± 19.7</td>
<td>49.2 ± 15.9</td>
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<tr>
<td>Insulin (U/kg/day)</td>
<td>40.4 ± 22.3</td>
<td>39.6 ± 20.5</td>
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<tr>
<td>HbA1c (%)</td>
<td>12.8 ± 3.2</td>
<td>12.4 ± 2.5</td>
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<tr>
<td>C Peptide (ng/ml)</td>
<td>0.9 ± 0.4</td>
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<tr>
<td>Diabetes duration (days)</td>
<td>21.4 ± 4.4</td>
<td>28.5 ± 7.5</td>
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Figure 1 - Flow diagram showing the progress of the patients throughout the trial
Figure 2 - HbA1c values (A) and relative change in HbA1c (B) throughout 24 week treatment and 12-week washout period for etanercept and placebo treated groups. Values represent mean ± SEM at each time point. * p ≤ 0.05; ^ p ≤ 0.01.
Figure 3 - C-peptide at baseline and 24-week for individual subjects (Etanercept solid circles; placebo open circles)