No Effect of the Altered-Peptide Ligand NBI-6024 on Beta Cell Residual Function and Insulin Needs in New-Onset Type 1 Diabetes

Running title: No effect of APL NBI-6024 on Type 1 Diabetes

Markus Walter, MD¹, Areti Philotheou, MD², François Bonnici, MD², Anette-G. Ziegler, MD¹, Roland Jimenez³, on behalf of the NBI-6024 study group*

*The NBI Study Group is listed in the Acknowledgements.

¹ Diabetes Research Institute, Forschergruppe Diabetes e.V., Munich, Germany; ² Diabetes Clinical Trials Unit, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa; ³ Clinical Development/Neurocrine Biosciences, Inc.

Corresponding Author:
Anette-G. Ziegler, MD
Email: anziegler@lrz.uni-muenchen.de

Clinical trial reg. no. NCT00873561, clinicaltrials.gov

Additional information for this article can be found in an online appendix at http://care.diabetesjournals.org

Submitted 6 April 2009 and accepted 3 August 2009.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
Objective - This randomized, four-arm, placebo-controlled dose-ranging phase-2 trial was conducted to determine whether repeated subcutaneous injections of the altered peptide ligand, NBI-6024, designed to inhibit autoreactive T-cells, improves beta cell function in patients with recently diagnosed type 1 diabetes.

Research, Design and Methods - One hundred eighty-eight patients, aged 10-35 years with recently diagnosed type 1 diabetes were randomized for a treatment consisting in the subcutaneous administration of placebo or 1 mg, 0.5 mg or 0.1 mg of NBI-6024 at baseline, week 2 and 4 and then monthly until month 24. Fasting, peak and AUC C-peptide concentrations during a 2-hour mixed meal tolerance test were measured at 3-monthly intervals during treatment. Immune function parameters (islet antibodies, CD4 and CD( T cells) were also studied.

Results The mean peak C-peptide at 24 months after study entry showed no significant difference between the groups treated with 0.1 mg (0.59 pmol/mL), 0.5 mg (0.57 pmol/mL), and 1.0 mg NBI-6024 (0.48 pmol/mL), and the placebo group (0.54 pmol/mL). Fasting, stimulated peak and AUC C-peptide concentrations declined linearly in all groups by approximately 60% over the 24 month treatment period. The average daily insulin needs at month 24 values were also comparable between the 4 groups. No treatment related changes in islet antibodies and T cell numbers were observed.

Conclusions Treatment with altered peptide ligand NBI-6024 at repeated doses of 0.1, 0.5 or 1.0 mg did not improve or maintain beta cell function.

Trial registration number at ClinicalTrials.gov: NCT00873561
Type 1 diabetes results from a T cell mediated autoimmune attack against the insulin-producing cells of the pancreatic islets (1-3). There is no curative treatment available to control these autoreactive T cells, rendering the patients dependent on insulin injections for normoglycemia. A treatment that could stop or reduce autoimmune destruction of pancreatic β cells would be a major advance in diabetic treatment and the potential prevention of diabetes in individuals genetically predisposed to developing the disease (4).

There is potential to target specific populations of autoreactive T-cells by identifying the dominant antigens responsible for their activation and producing a soluble altered peptide ligand (APL) to block or change this response. The insulin B(9-23) peptide has been shown to be an important antigen of T cells in autoimmune diabetes in animals and man (5). NBI-6024 is an altered peptide ligand (APL) and contains two natural L-amino acid substitutions in the (9-23) sequence of the B-chain of insulin. Alanine is substituted for tyrosine at position 16, which is a key contact site at the T-cell receptor, and at position 19 for cysteine. The resulting APL (Ala16,19), known as NBI-6024, does not activate insulin B(9-23)-reactive murine or human T-cells (6). Nonobese diabetic mice treated with NBI-6024 are protected from developing diabetes, even though other T cells with different antigenic specificities were present, suggesting that the immune response induced by the APL may regulate pathogenic T cells through the production of regulatory cytokines such as IL-4 (6).

Preliminary results of three studies in adult male patients with type 1 diabetes had indicated that NBI-6024 administration is safe and well tolerated (7,8). To investigate the pharmacologic potential of NBI-6024 to improve beta cell function, a multicenter, randomized, four-arm, placebo-controlled phase-2 trial was performed. The primary objective of the trial was to assess the effect of repeated administrations of NBI-6024 on endogenous insulin production as measured by C-peptide concentration in adult and adolescent patients with recent onset type 1 diabetes. Insulin usage, glycemic control, and immune function were also assessed.

**RESEARCH, DESIGN AND METHODS**

Patients with recent onset type 1 diabetes were selected according to the following criteria: they were 10 to 17 years of age (adolescent group) or 18 to 35 years of age (adult group), had symptoms for no longer than 6 months, had been treated with insulin for less than 3 months, had a positive result on testing for islet autoantibodies (antiGAD antibodies or anti-ICA512 antibodies, or anti-insulin antibodies provided that the patient was not on insulin therapy for more than 2 weeks), had a stimulated C-peptide peak concentration between 0.4pmol/mL and 3.0pmol/mL, had a body mass index < 28 kg/m2, laboratory and ECG results within normal ranges, and compliance with insulin treatment. Pregnant or lactating women were excluded, and female patients with childbearing potential had to practice an acceptable contraceptive technique from 30 days before enrolment until 30 days after the last dose of study drug. Written informed consent was obtained from each patient. The trial was approved by the ethics committee at each center.

**Study Centers.** A total of 22 centers participated in the study including
six centers in South Africa (103 patients randomized), one in the UK (3 patients), two in the Czech Republic (23 patients), four in Spain (10 patients), one in Finland (5 patients), two in Germany (33 patients), four in Canada (9 patients), and two in France (2 patients).

**Study Design.** The trial was performed as a phase II, multicenter, randomized, double-blind, placebo-controlled, parallel, dose-ranging study between 2001 and 2006. Of 266 patients screened, a total of 188 patients were randomized, including 111 adolescents (figure 1). Eligible patients were randomized in a 1:1:1:1 ratio to one of the following treatments: NBI-6024 0.1 mg (N=50), 0.5 mg (N=48), or 1 mg (N=43), or placebo (N=47). On Day 1, patients received their first dose of study drug and were discharged approximately 2 hours post dose at the discretion of the investigator. Study drug was administered subcutaneously for a total of 26 times over a 24-month period. The first three doses were administered every 2 weeks (induction phase); all subsequent dosing occurred monthly (maintenance phase). Dose and dosing frequency were selected after considering immune efficacy and tolerability in animal studies. Patients returned to the study center to receive study drug and have efficacy and safety assessments performed. Patients continued on their normal insulin regimen throughout the study, unless changes were clinically indicated. In order to avoid possible confounding through differences in glycemic control among the groups, diabetes management and glycemic targets were standardized as much as possible in all patients. Patients with HbA1c levels above 8% had additional contacts with the investigator in order to improve their metabolic control. All patients were treated with intensive insulin therapy.

**Endpoint assessments.** The primary efficacy variable was the 2-hour peak C-peptide level at 24 months. A mixed meal tolerance test (MMTT; Boost® High Protein from Novartis in an amount of 6 mL per kg body weight, up to 360 mL) was administered to collect stimulated C-peptide samples (0, 30, 60, 90 and 120 minutes) every three months and at the follow-up visit. Secondary efficacy variables were the area under the curve (AUC 0-120 minutes) C-peptide at 24 months, the prescribed insulin usage and the glycemic control, assessed by HbA1c levels, and the hypo- and hyperglycemic events documented in the patient’s diary. C-peptide concentrations and HbA1c were measured at a central laboratory (ICON Laboratories, NY, USA) by using radioimmunoassay (Diagnostic Systems Laboratories) and high pressure liquid chromatography, respectively. The reported inter-assay CV of the C-peptide assay is 5.3% at a concentration of 0.55 nmol/L and the lower limit of detection is 0.03 nmol/L.

**Safety parameters and hypoglycaemic events.** Safety parameters included vital signs (body temperature, supine heart rate, blood pressure) before and one hour after study drug administration, laboratory tests (haematology, clinical chemistry, urinalysis with microscopy) and quarterly physical examinations as well as ECG and documentation of adverse events and concomitant medication. Hypoglycemic events were defined as any blood glucose level below 50 mg/dL (3 mmol/L) with or without typical symptoms, or typical symptoms if the blood glucose level was 50 mg/dL or higher. If the patient required intervention
by another person, the event was defined as a “major hypoglycaemic event”.

**Immune markers.** CD4 and CD8 T cell numbers were measured by flow cytometry on whole blood using a standard protocol at a central laboratory (ICON Laboratory, Dublin, Ireland). Antibodies to insulin, GAD65, and IA-2 were measured by ICON Laboratories (NY, USA) using the Kronus radiobinding assays (Boise, USA) according to manufacturer’s instructions.

**Statistical Methods.** All data were summarized by treatment group. All randomized patients were included in the modified intent-to-treat (ITT) population. All randomized patients who had taken at least one dose of study drug and had some post-baseline data for the primary efficacy parameter (2-hour peak C-peptide) were included in this population. The efficacy evaluable (EE) population included patients in the ITT population who received at least 13 doses and did not have any major protocol deviations. The safety population was defined as all randomized patients who received at least one dose of study drug. All hypotheses testing were 2-sided and carried out at the 5% significance level.

**RESULTS**

Of 188 patients randomized, 168 completed the study (figure 1). The 20 patients who dropped out from the study included 12 who withdrew consent, three who had an adverse event, two who became pregnant, and three who discontinued for other reasons. The four treatment groups were similar in terms of age, duration of disease, ethnicity, and baseline C-peptide concentrations (table 1).

**Safety parameters.** Adverse events (AE) reported by ≥10% of patients were upper respiratory tract infection, nasopharyngitis, headache, pharyngitis, influenza, nausea, rhinitis, and vomiting. There was no clear pattern of relationship between dose, occurrence, and frequency of AE. The frequencies of AE in the NBI-6024 treatment groups and the placebo group were comparable. There was no clinically significant change in laboratory or vital sign parameters. Overall, 39 (20.7%) patients were reported as experiencing at least one serious adverse event (SAE); none of the SAEs was considered drug-related.

**Efficacy parameters.** The mean peak C-peptide at month 24 and the change in the mean values from baseline to month 24 were comparable in the NBI-6024 treatment groups and the placebo group in the ITT as well as the EE population (ITT population: mean peak C-peptide at 24 months (0.1mg: 0.39 pmol/mL, 0.5mg: 0.39 pmol/mL, 1mg: 0.33 pmol/mL, placebo: 0.39 pmol/mL, p values 0.4, 0.3 and 0.6; figure 2). Similarly, the mean AUC of C-peptide at baseline and at month 24 in the NBI-6024 treatment groups and in the placebo group were comparable (table 2).

The average daily insulin need at 24 months was 47.8 IU (20.4 SD), 57.2 IU (36.5 SD), and 51.6 IU (21.8 SD) in the 0.1mg, 0.5mg, 1mg NBI-6024 treatment groups, respectively, and 47.3 IU (17.6 SD) in the placebo group (p values 0.9, 0.05 and 0.2) (figure 3).

The HbA1c values at months 24 were slightly higher in the NBI-6024 treatment groups compared to the placebo group (0.1mg: 8.1 (2.1 SD), 0.5mg: 8.8 (3.0 SD), 1mg: 8.6 (2.6 SD), placebo: 8.0 (2.1 SD); p values 0.7, 0.01, 0.1) (figure 3). The number of patients who reached an HbA1c below 7% at 24 months were 34%, 32%, 35% and 29% in the NBI-6024 treatment groups and the
placebo group, respectively (p values 0.6, 0.9 and 0.6).
When stratifying the analysis by age, no significant difference was found for peak C-peptide at month 24 as well for the decline in peak C-peptide from baseline to 24 months between adolescent patients and adults (figure 2). Furthermore, there was no effect of gender or ethnicity.

**Hypoglycemic events.** Most hypoglycaemic events were deemed to be minor events. A pairwise treatment comparison showed no statistically significant difference between NBI-6024 treatment groups compared to placebo in the monthly rate of hypoglycaemic events (mean monthly rate (SD): 0.1mg 1.9 (2.0), 0.5mg 1.7 (1.5), 1mg 1.9 (1.6), placebo 1.8 (2.4), p values 0.6, 0.3 and 0.4). About 9% of patients experienced a major hypoglycaemic event during this study; these patients were evenly distributed across treatment groups.

**Immune function parameters.** Insulin antibodies increased in concentration from the baseline visit to month 1 and remained stable thereafter in all groups. No differences in antibodies to insulin, GAD65 and IA-2 were observed between treatment and placebo groups at all study visits (see supplementary Figure S1 in the online appendix which is available at [http://care.diabetesjournals.org](http://care.diabetesjournals.org)). No differences were observed for CD4 and CD8 peripheral blood T cell numbers between treatment and placebo groups (supplementary Figure S2).

**CONCLUSION**

For the first time, an altered peptide ligand (APL) was used for treatment of autoimmune diabetes in a trial that included 188 patients with recent onset disease. The APL NBI-6024, at the doses studied, was well tolerated. There were no significant safety issues and most adverse events were considered mild or moderate, and unrelated or unlikely related to study drug. The frequency of injection site reactions, a surrogate marker of immunoreactivity, was comparable among placebo and active treatment groups. Treatment with NBI-6024 at the doses studied, however, provided no protection against the decline of β-cell function after diabetes onset, as measured by fasting or stimulated C-peptide. Moreover, it did not cause significant changes in insulin requirement, metabolic control, hypoglycemic and hyperglycemic events, autoantibody concentrations or CD4 and CD8 T cell numbers.

The NBI-6024 clinical trial was an intensively monitored intervention study that collected data on C-peptide, glucose, and HbA1c every 3 months for 24 months with central measurement of these variables. It provided a detailed natural history of beta cell function after diabetes onset in unprecedented numbers of well characterized and selected adolescents and adults with recent onset type 1 diabetes. One limitation of the current trial is that no T cell response data was obtained to verify an immunological effect at the doses given. However, the study included 3 doses over a 10-fold range which had previously been shown to cause T cell unresponsiveness. The lack of response may reflect a fundamental defect in the proposed mechanism of action or inadequacy of exposure (dose), frequency or timing of the investigational drug.

With respect to the natural history of beta cell function, the study suggests a linear continuous decline of beta cell function over a period of two years after disease onset. The rate of decline was similar between adolescents and adults.
with slope estimates of -0.034 and -0.031 respectively for peak c-peptide values. All three parameters of beta cell function examined – fasting c-peptide, peak c-peptide, and AUC of c-peptide response in the mixed meal test showed similar declines with an overall loss of around 30% by 12 months of follow-up and 60% by 24 months of follow-up. This data is likely to be useful as reference for other trials and for future trial planning. For comparison, there was 50-60% decline in fasting and stimulated c-peptide concentrations within 21 months in the recently reported GAD-alum vaccination trial in patients aged 10 to 18 years (9).

In summary, this study failed to demonstrate efficacy of APL treatment in patients at the stage of overt disease. The continuous decline of beta cell function after onset, however, provides important data for design and validation of future clinical trials in patients with type 1 diabetes and confirms the need of novel treatment strategies (10) to reduce this progressive beta cell loss.

Acknowledgements

**NBI Study group:** South Africa: L. Distiller, A. Philotheou, R. Moore, L.I. Robertson, J. Wing, G. Ellis; B.D. Kramer, F.A. Mahomed, B.I. Joffe, F. Bonnici, P.J. Wormald, S. Brown, A. Murphy, N. Gurtunca, L. Hofmann, M.-T. van der Merve, S. Goldburg, H. Wellmann. United Kingdom: S. Greene, V. Franklin. Czech Republic: J. Vavrinec, J. Venhacova, Z. Sumnik, S. Kolouskova, O. Cinek, K. Stechova, P. Venhacova. Spain: R. Gomis, P. Martul, J.P. López-Siguero, D. Acosta-Delgado, I. Conget, E. Agullera, J.A. Vazquez Garcia, L. Castaño, F. Vazquez San Miguel, I. Rica, V. Martín, MªS. Ruiz de Adana Navas, A. del Pino de la Fuente, M.J. Garcia Arias, M.A. Mangas Cruz, R. Guerrero. Finland: I. Sipilä, T. Saukkonen, P.J. Miettinen, T. Otonkoski. Germany: A.-G. Ziegler, T. Danne, M. Füchtenbusch, M. Walter, T. Kaupper, C. Bittner, W. von Schutz, A. Krautzig, A. Hackenberg, N. Datz. Canada: A. Belanger, P. Perron, D. Pacaud, M. Lawson, J. Palary, D. Kandalaft, R. Duma, M.F. Langlois, K. Khoury, J.P. Baillargeon, S. Lawrence, A. Hadjiyannakis. France: P.-F. Bougnères, M. Nicolino, M.C. Andre, J.C. Carel, P.P. Boileau, M. François, N. Bendelac, F. Texier, P. Bretones, C.-L. Gay.

We thank the Neurocrine Biosciences Inc. Development Team for their efforts, i3 Research for study management, all technicians and study nurses who contributed to this project and all patients who participated in the study.

**Disclosure** - Roland Jimenez is part of the Neurocrine Biosciences Inc. Development Team and director of the Clinical GnRH Program of Neurocrine Biosciences Inc. All other authors have no conflicts of interest or relevant financial interests. Anette Ziegler and Markus Walter had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
No effect of APL NBI-6024 on Type 1 Diabetes

References
1. Eisenbarth GS: Type 1 Diabetes Mellitus: A Chronic Autoimmune Disease. *New Engl J Med* 314:1360-1368, 1986
2. Roep BO, Atkinson MA, van Endert PM, Gottlieb PA, Wilson SB, Sachs JA: Autoreactive T cell responses in insulin-dependent (type 1) diabetes mellitus. *J Autoimmun* 13:267-282, 1999
3. Lejon K, Fathman CG: Isolation of self antigen-reactive cells from inflamed islets of nonobese diabetic mice using CD4 high expression as a marker. *J Immunol* 163:5708-5714, 1999
4. Stiller CR, Dupre J, Gent M, Jenner MR, Keown PA, Laupacis A, Martell R, Rodger NW, von Graffenried B, Wolfe BM: Effects of Cyclosporine Immunosuppression in Insulin-Dependent Diabetes Mellitus of Recent Onset. *Science* 223:1362-1367, 1984
5. Wegmann DR, Gill RG, Norbury-Glaser M, Schloot N, Daniel D: Analysis of the spontaneous T cell response to insulin in NOD mice. *J Autoimmun* 7:833-843, 1994
6. Alleva DG, Gaur A, Jin L, Wegmann D, Gottlieb PA, Pahuja A, Johnson EB, Motheral T, Putnam A, Crowe PD, Ling N, Boehme SA, Conlon PJ: Immunological characterization and therapeutic activity of an altered-peptide ligand, NBI-6024, based on the immunodominant type 1 diabetes autoantigen insulin B-chain (9-23) peptide. *Diabetes* 51:2126-2134, 2002
7. Giannoukakis N. NBI-6024 (Neurocrine Biosciences). *IDrugs* 5:1162-1167, 2002
8. Alleva DG, Maki RA, Putnam AL, Robinson JM, Kipnes MS, Dandona P, Marks JB, Simmons DL, Greenbaum CJ, Jimenez RG, Conlon PJ, Gottlieb PA: Immunomodulation in type 1 diabetes by NBI-6024, an altered peptide ligand of the insulin B epitope. *Scand J Immunol* 63:59-69, 2006
9. Ludvigsson J, Faresjö M, Axelsson S, Chéramy M, Pihl M, Vaarala O, Forsander G, Ivarsson S, Johansson C, Lindh A, Nilsson NO, Aman J, Ortgvist E, Zerhouni P, Casas R: GAD Treatment and Insulin Secretion in Recent-Onset Type 1 Diabetes. *N Engl J Med* 359:1909-1920, 2008
10. Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, Gorus F, Goldman M, Walter M, Candon S, Schandene L, Crenier L, De Block C, Seigneurin JM, De Pauw P, Pierard D, Weets I, Rebell P, Bird P, Berrie E, Frewin M, Waldmann H, Bach JF, Pipeleers D, Chatenoud L: Insulin Needs after CD3-Antibody Therapy in New-Onset Type 1 Diabetes. *N Engl J Med* 352:2598-2608, 2005
Table 1. Patient Demographics and History of Diabetes by Treatment Group

| Treatment Group | NBI-6024 0.1 mg (N = 50) | NBI-6024 0.5 mg (N = 48) | NBI-6024 1.0 mg (N = 43) | Placebo (N = 47) |
|-----------------|--------------------------|--------------------------|--------------------------|------------------|
| Age (years)     | Mean (SD)                | 17.9 (6.3)               | 17.4 (6.4)               | 17.9 (6.4)       | 18.7 (7.1)       |
| Gender (N)      |                          |                          |                          |                  |
| Male            |                          | 31                       | 26                       | 30               | 31               |
| Female          |                          | 19                       | 22                       | 13               | 16               |
| Peak C-peptide baseline (pmol/mL) | Mean (SD) | 1.36 (0.63) | 1.35 (0.63) | 1.41 (0.63) | 1.42 (0.61) |
| HbA1c baseline (%) | Mean (SD) | 7.9 (1.6)    | 7.8 (1.6)    | 8.1 (2.0)      | 8.1 (2.0)       |
| Daily insulin dose (IU) | Mean (SD) | 31.3 (19.4) | 33.1 (15.8) | 30.5 (11.9) | 32.1 (14.9) |
| Time since symptoms (months) | Mean (SD) | 2.1 (1.1)    | 2.1 (1.4)    | 2.1 (1.1)       | 2.3 (1.9)       |
| Time since diagnosis (months) | Mean (SD) | 1.2 (0.5)    | 1.2 (0.7)    | 1.2 (0.7)       | 1.2 (0.8)       |

TABLE 2. AUC\textsubscript{0-120 MIN} C-PEPTIDE (PMOL × MIN/ML) BY TREATMENT GROUP

| Treatment Group | NBI-6024 0.1 mg (N = 47) | NBI-6024 0.5 mg (N = 48) | NBI-6024 1.0 mg (N = 43) | Placebo (N = 40) |
|-----------------|--------------------------|--------------------------|--------------------------|------------------|
| Baseline        | N                        | 47                       | 48                       | 43               | 44               |
| Mean (SD)       | 134 (63)                 | 131 (62)                 | 135 (57)                 | 134 (59)         |
| Month 24        | N                        | 42                       | 46                       | 37               | 40               |
| Mean (SD)       | 57 (71)                  | 53 (56)                  | 44 (55)                  | 50 (45)          |
| p-value         | 0.5                      | 0.6                      | 0.9                      |

Figure Legends:

Figure 1. Study Participation and Randomization

Figure 2. Mean fasting C-peptide levels (a) and mean peak C-peptide levels after mixed meal stimulation in the four treatment groups [all subjects (b), adolescents (c), adults (d)]. No significant difference in C-peptide levels was observed between treatment groups. The decline in C-peptide secretion from baseline to 24 months was similar between adolescent patients and adults.

Figure 3. Mean HbA1c values (a) and mean daily insulin requirement (b) in the four treatment groups.
No effect of APL NBI-6024 on Type 1 Diabetes

Figure 1

Number of patients screened
N = 266

Number of non-randomized patients
N = 78

Number of randomized patients
N = 188

NBI-6024 0.1mg
N = 50

NBI-6024 0.5mg
N = 48

NBI-6024 1.0mg
N = 43

Placebo
N = 47

Completed study
N = 42

Completed study
N = 47

Completed study
N = 39

Completed study
N = 40

Figure 2

a) Fasting C-Peptide – All Subjects

b) Peak C-peptide – All Subjects

C) Peak C-peptide – Adolescents

d) Peak C-peptide – Adults
No effect of APL NBI-6024 on Type 1 Diabetes

Figure 3

a  HbA1c Over Time - All Subjects

b  Insulin—Average Daily Dose