Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass

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Abstract

Aims/hypothesis The aim of the study was to examine the 48 month outcome of treating recent-onset type 1 diabetic patients for 6 days with humanised CD3-antibody, ChAglyCD3.

Methods Eighty patients, aged 12–39 years, were recruited for a phase 2 multicentre trial and randomised to placebo ($n=40$) or ChAglyCD3 ($n=40$) treatment by a third party member; participants and care-givers were blinded. The change in insulin dose ($\text{U kg}^{-1} \text{day}^{-1}$) over 48 months was chosen as primary endpoint and compared in 31 placebo- and 33 ChAglyCD3-treated patients. Adverse events were followed in 35 and 38 patients, respectively.

Results Treatment with ChAglyCD3 delayed the rise in insulin requirements of patients with recent-onset diabetes and reduced its amplitude over 48 months ($+0.09$ vs $+0.32 \text{ U kg}^{-1} \text{day}^{-1}$ in the placebo group). Using multivariate analysis this effect was correlated with higher baseline residual beta cell function and a younger age. It was associated with better outcome variables in subgroups selected according to these variables. In the ChAglyCD3 subgroup with higher initial

beta cell function, 0/11 patients became C-peptide-negative over 48 months vs 4/9 in the corresponding placebo subgroup. In the subgroup aged <27 years old, antibody treatment preserved initial beta cell function for 36 months (vs $>80\%$ decline within 24 months in the placebo subgroup <27 years old), resulted in lower HbA_{1c} concentrations and tended to reduce glycaemic variability ($p=0.08$). No long-term adverse events were observed.

Conclusions/interpretation A 6 day ChAglyCD3 treatment can suppress the rise in insulin requirements of recent-onset type 1 diabetic patients over 48 months, depending on their age and initial residual beta cell function. In younger patients this effect is associated with reduced deterioration of metabolic variables. These observations help to define inclusion criteria for prevention trials.

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Keywords Antibody treatment · C-peptide · Diabetes · Endocrine pancreas · Hypoglycaemia · Insulin · Islet

Abbreviations

EBV Epstein–Barr virus
FBG Fasting blood glucose
ICA Islet-cell autoantibodies

Introduction

Studies in rodents have shown that CD3-specific antibodies can induce prolonged remission of established autoimmunity by restoring immune tolerance [1]. A short course in non-obese diabetic (NOD) mice, treated within 1 week after onset, induced a long-lasting normalisation of the hyperglycaemic state and an inhibition of beta cell-specific immune activities without affecting responses to unrelated antigens [2]. Similar effects were noted in experimental allergic encephalomyelitis, a model of multiple sclerosis [3]. This treatment was also tested in recent-onset type 1 diabetic patients using humanised Fc mutated anti-human CD3 antibodies [4–6], which are less mitogenic and cytokine-releasing than murine FcR-binding CD3 antibodies such as murinomab CD3 (orthoclone OKT3) [7–9]. In a Phase 1/2 trial, a CD3-specific monoclonal antibody, hOKT3 γ 1 (Ala-Ala), was administered for 2 weeks [4, 6]. In our Phase 2 placebo-controlled trial, the aglycosylated recombinant ChAglyCD3 was administered for six consecutive days [5]. In both studies, an improved average beta cell function and lower average insulin dosage was seen in antibody-treated groups for at least 18 months [5, 6]. The placebo-controlled trial showed that this protective effect was most pronounced

in the subgroup of patients with initial residual beta cell function at or above the 50th percentile of the 80 patients included [5]. In the present 48 month follow-up study of placebo- and antibody-treated groups, we followed this effect further to examine its correlation with baseline characteristics and its association with metabolic variables. We also assessed whether antibody treatment caused long-term adverse events.

Methods

Patient selection and treatment Eighty recent-onset type 1 diabetic patients were included in a double-blind randomised phase 2 placebo-controlled trial [5]. They were selected according to age (12–39 years), positivity for islet-cell autoantibodies (ICA) and/or glutamic acid decarboxylase autoantibodies, random C-peptide >0.2 nmol/l (at glycaemia 10.0–13.9 mmol/l), insulin treatment for <4 weeks, polyuria for <6 months, weight loss <10% during the previous 6 months, and positivity for Epstein–Barr virus (EBV) IgG. Forty patients received i.v. ChAglyCD3 on consecutive days and 40 received a placebo. Treatment allocation by a third party was randomised according to trial centre (four in Belgium, one in Germany), age (< or $\geq$ 15 years) and presence or absence of ICA. The initial protocol had been planned for an 18 month study, with data reported by Keymeulen et al. [5]. After this period, patients re-consented to a follow-up study till month 48. Both the initial and extension study were approved by the Belgian Diabetes Registry and by the ethics committee of each participating centre. Written informed consent was obtained from each patient.

Of the initial 80 patients, 73 re-consented to the extension study (35 in the placebo group and 38 in the ChAglyCD3 group; characteristics of the participants are shown in Table 1 and the study design in Fig. 1). Reasons for not participating were withdrawal from further regular testing (placebo, $n=2$), a stay abroad (ChAglyCD3, $n=3$) and pregnancy (ChAglyCD3, $n=1$). Both groups had demographics and baseline characteristics similar to the population at start of the study, except for a slightly higher HbA_{1c} level in the ChAglyCD3 extension group ($p=0.02$) (Table 1). There were no differences in medication with effects on insulin needs; in both groups three patients temporarily received corticoids during follow-up (Electronic supplementary material [ESM] Table 1).

Patient follow-up Patients were on intensive insulin therapy for the entire 48 month period. At least once every 3 months, insulin doses were adjusted by the patient's diabetologist to achieve HbA_{1c} levels <7% as part of

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Table 1 Characteristics of patients that participated in the extension study

Variable	Placebo group (n=35)	ChAglyCD3 group (n=38)
Demographics		
Sex (male/female)	23/12	25/13
Age (years)	26±7	28±6
At start of treatment (baseline)		
Time since diagnosis (days)	25 (19–27)	21 (18–24)
Time of insulin treatment (days)	18±9	19±5
Insulin dose (U/day)	26±14	31±17
Insulin dose (U kg ⁻¹ day ⁻¹)	0.39±0.21	0.46±0.28
Body weight (kg)	68±12	71±15
Body mass index (kg/m ²)	21.2 (19.9–23.9)	22.1 (20.4–24.0)
HbA _{1c} (%)	8.1±1.3	8.8±1.6
ICA≥12 JDF (%)	71	79
GADA≥2.6 (%)	97	84
C-peptide release under glucose clamping (nmol l ⁻¹ min ⁻¹)		
Without glucagon	0.63±0.30	0.55±0.31
With glucagon	0.96±0.45	0.86±0.46
Use of hypoglycaemic drugs		
ACE inhibitors ^a	0 (2)	0 (1)
Metformin ^a	1 (1)	0 (2)

Data represent means±SD (in case of normal distribution) or median with interquartile range (in case of non-normal distribution)

^aNumber of patients on treatment at baseline (at month 48)

routine care; for 73% of patients, their diabetologist did not participate in the study. Patients adjusted pre-prandial insulin doses by carbohydrate counting and home glucose monitoring, targeting pre-prandial glycaemia at 4.4–7.8 mmol/l.

Outpatient study visits occurred at months 24±3, 36±3 and 48±3 at the trial centre or at the local hospital. Body weight, type and dose of insulin, home blood glucose measurements, concomitant medication and adverse events were recorded. Blood samples were taken for determination of HbA_{1c}, and residual beta cell function was measured through C-peptide release during a glucose-clamp [5, 10, 11]. The clamp was not carried out in patients who were C-peptide-negative at a previous visit; a zero value was filled in for subsequent time points and included in the analysis. Clamps consisted of a 180 min euglycaemic phase (3.3–5.0 mmol/l) followed by 146 min at 10.0–13.9 mmol/l with 1 mg glucagon injected at minute 140 [5]. C-peptide release was expressed as the AUC during sustained glucose stimulation in absence (between minutes 60 and 140) and presence (between minutes 140 and 146) of glucagon. C-peptide-negativity was defined as AUC C-peptide ≤0.03 nmol/l×min during hyperglycaemic clamp. In Belgian trial centres flow cytometry was used to quantify peripheral lymphocyte subsets. [12]

Statistical analysis Data were stored at the Belgian Diabetes Registry and analysed by a statistician (L. Kaufman)

who was not a member of the trial team. The extension study followed all variables that had been predefined for the 18 month study [5]. We did, however, change the primary endpoint (changes in baseline residual beta cell function) because only 36 of the 73 re-consented patients consented to continue clamp testing. It was replaced by changes from baseline in insulin dose/kg body weight. At baseline as well as at month 18, this variable showed a strong correlation with residual beta cell function. Evaluation of efficacy, and its multivariate analysis, was carried out on all patients for whom insulin dose and body weight were available at baseline and at 48 months. This was the case in 33 ChAglyCD3-treated and 31 placebo patients (Fig. 1). In these patient groups, 21 and 15, respectively, had re-consented to clamp tests (Fig. 1). One patient refused a clamp test at month 48 while he had remained C-peptide-positive till month 36; the clamp analysis at month 48 was thus conducted on 21 and 14 patients, respectively. Patients becoming C-peptide-negative during the study remained included in the clamp analysis with zero values filled in for the time points of C-peptide-negativity.

Multivariate analyses using stepwise multiple linear regression were performed in each group and in both groups together in order to investigate correlations between changes in insulin dose over 48 months and baseline variables. Before start of treatment, the following potentially prognostic baseline variables had been defined: age, sex, body weight and BMI, HLA-DQ genotype (protective;

Fig. 1 Study design

not protective), residual beta cell function, HbA_{1c}, ICA (<12 JDF; ≥12 JDF), GADA (<2.6%; ≥2.6%) and baseline insulin dose (see Keymeulen et al. [5] and its supplementary appendices). A post hoc exploratory evaluation was carried out in which the effect of treatment was assessed in subgroups that differed for two of these predefined variables, namely baseline residual function (AUC C-peptide 60–140 min <50th percentile and ≥50th percentile) or age (<50th percentile and ≥50th percentile). This evaluation was performed for each time-point using the Mann–Whitney *U* test. Adjustments were made for multiplicity. All statistical tests were performed two-sided at the 5% level of significance. In the efficacy and multivariate analysis, country was added as variable since patients in Germany and their diabetologists were, from month 18 onwards, aware of their treatment allocation, while Belgian patients and physicians were not. In the ChAglyCD3 group 12/33 patients and in the placebo group 8/31 were German ($p=0.43$).

Results

Effect of ChAglyCD3 on insulin requirements over 48 months

Mean daily insulin dose steadily increased from the start of the trial in the placebo group ($+0.32 \text{ U kg}^{-1} \text{ day}^{-1}$ in 48 months) while it initially decreased in the ChAglyCD3 group and then increased only slightly above start values ($+0.09 \text{ U kg}^{-1} \text{ day}^{-1}$ in 48 months) (Fig. 2a). At all time points, changes in insulin dose vs baseline were significantly different between both groups. The lower mean insulin doses after ChAglyCD3 were not caused by under-treatment since the corresponding HbA_{1c} levels were not higher than in the placebo group (Fig. 2b). They were associated with a higher residual beta cell function as measured by the AUC during glucose clamp with glucagon (Fig. 2c). Mean beta cell function declined rapidly and steadily after placebo, but it was maintained for at least 24 months after ChAglyCD3 treatment ($p>$

Fig. 2 Efficacy tests in all patients and in subpopulations during 48 month follow-up. Blue circles represent the placebo group and red triangles the ChAglyCD3 group. Daily insulin needs (**a**), HbA_{1c} levels (**b**) and residual beta cell function measured as C-peptide release during glucose clamp plus glucagon (**c**) are shown for all patients. In panels **d–f** these values are ranked according to whether residual beta cell function at baseline was below or at or above the median (50th percentile) of all 80 patients at baseline. The 50th percentile was approximately 75% lower than the median value measured in age-matched control participants. In panels **g–i** these values are ranked according to whether age at baseline was below or at or above the median (50th percentile) of all 80 patients at baseline. The 50th

percentile was at age 27 years. Values represent means±SE. Statistical difference in the change from baseline between both groups: * $p<0.05$, ** $p<0.01$, *** $p<0.001$. In order to correct for multiple testing, data were also interpreted according to a Bonferroni procedure. Since five comparisons were made (6, 12, 24, 36 and 48 months), results are considered statistically significant if $p<0.01$ (0.05/5). Based on this correction, significant differences in the changes from baseline insulin dose were maintained between both groups at 6, 24, 36 and 48 months (all patients). For each variable and for each time point the number of patients for whom data were available is shown. Statistical difference between subgroups: † $p<0.05$, †† $p<0.01$, ††† $p<0.001$

0.05 vs baseline). At month 36, residual beta cell function in the ChAglyCD3 group was 80% higher than in the placebo group. Over the 48 month follow-up period, fewer ChAglyCD3-treated patients had become C-peptide-negative (4/21 vs 7/14 placebo patients, $p=0.07$). The number of severe hypoglycaemic events was low in both

groups ($n=3$ in the placebo group and $n=1$ in the ChAglyCD3 group), making it an insensitive variable for assessing differences in metabolic outcome at this stage.

Correlation between the effect of ChAglyCD3 on daily insulin requirements and baseline variables In multivariate

analysis two baseline variables were independently correlated with the effect of the antibody treatment on daily insulin requirements over 48 months. Analysis of the insulin dose ($\text{U kg}^{-1}\text{day}^{-1}$) at month 48 showed that age was a significant factor in the CD3 group ($p=0.009$) and baseline residual beta cell function in the placebo group ($p=0.004$). When performing this analysis on both groups together, both age ($p=0.004$) and baseline residual beta cell function ($p=0.019$) were statistically significantly correlated, as was antibody treatment ($p=0.005$).

On basis of this finding we conducted two post hoc analyses in which patients were subdivided according to one of these baseline variables, i.e. baseline residual beta cell function (C-peptide release with glucose clamping) or age. Each subdivision was then made into two categories on the basis of values below or above the 50th percentile that had been determined in the 80 patients at start of the study. Categories were denoted as initial C-peptide release <50 th percentile and ≥ 50 th percentile (Fig. 2d–f) and as age <50 th percentile and age ≥ 50 th percentile (Fig. 2g–i). Age categories were from 12 to 27 years and from 27 to 39 years, respectively.

Influence of beta cell function at baseline The ChAglyCD3-treated subgroup with initial C-peptide release ≥ 50 th percentile needed significantly lower insulin doses than the corresponding placebo subgroup (Fig. 2d). In the placebo subgroup, insulin requirements more than doubled during the first 24 months, while they stabilised at or below starting doses in the antibody-treated subgroup. Between months 24 and 48, mean insulin dose in the ChAglyCD3 subgroup increased progressively but remained 64–40% lower than after placebo. In the subgroups with initial C-peptide release <50 th percentile, no statistically significant differences were found in the time course of insulin requirements after placebo or ChAglyCD3 treatment ($p>0.15$ at all time points).

In the total group, the lower mean insulin doses in the ChAglyCD3 subgroup ≥ 50 th percentile were not caused by inadequate dosing (Fig. 2e), but appeared to be related to higher values for mean residual beta cell function (Fig. 2f). However, the clamp test data were no longer statistically higher during the extension study, probably due to a combination of a waning effect and the small number of patients that had agreed to a continued clamp test (Fig. 2f). Nevertheless, a persistence of a higher beta cell function in the antibody-treated subgroup may explain the lower number of patients that became C-peptide-negative during the 48 month follow-up in this group (0/11 vs 4/9 in the corresponding placebo subgroup; $p=0.03$). In patients with lower baseline beta cell function (C-peptide release <50 th percentile), residual beta cell function was never higher following antibody treatment and the number of C-peptide-

negative patients not lower (4/10 vs 3/5 in the placebo group; $p=0.61$).

Influence of age In the older subgroup (age 27–39 years), the antibody effect on mean insulin doses was only statistically significant during the first 24 months (Fig. 2g). The change in mean HbA_{1c} levels vs baseline was similar to that in the older placebo subgroup ($p>0.39$ at all time points; Fig. 2h). The antibody-treated and the placebo subgroups exhibited a similar residual beta cell function over the entire period, undergoing a 50% reduction over 48 months (Fig. 2i).

In the younger subgroup (age 12–27 years), ChAglyCD3 treatment resulted in 30–40% lower insulin doses than placebo at months 24, 36 and 48; at month 48, the dose was barely higher than at baseline ($+0.03$ vs $+0.36 \text{ U kg}^{-1}\text{day}^{-1}$ after placebo, $p=0.001$) (Fig. 2g). This subgroup had better HbA_{1c} levels (Fig. 2h). These antibody-induced effects were associated with a preservation of the initial beta cell function for at least 36 months, which contrasts with a $>80\%$ decline during the first 24 months after placebo (Fig. 2i). At month 48, mean beta cell function in the ChAglyCD3 subgroup had decreased by 50% but was still fivefold higher than in the placebo subgroup, although not reaching statistical significance. Similar differences were seen during the glucose clamp phase in absence of glucagon (ESM Fig. 1).

Since those in the younger age group had the most pronounced antibody-induced preservation of beta cell function, we used a post hoc analysis to examine whether this effect was associated with better glucose control as reflected by a greater number of patients with HbA_{1c} levels $<6.5\%$ [13], with fasting blood glucose (FBG) $<7.2 \text{ mmol/l}$ [14] and with CV for FBG of $<20\%$ [15]. Table 2 shows data at start of the study and at month 48, together with the number of patients that injected $<0.5 \text{ U kg}^{-1}\text{day}^{-1}$ insulin at these time points; data are also shown for the older age group. Comparison of the two placebo subgroups at month 48 shows that a better metabolic control was achieved in the older subgroup (9/16 with $\text{HbA}_{1c} <6.5\%$ vs 1/15 in the younger subgroup, $p<0.05$); this subgroup also had more patients with FBG $<7.2 \text{ mmol/l}$ and with CV for FBG of $<20\%$ (Table 2), but the low number of patients limits statistical analysis. Antibody treatment improved metabolic control in the younger subgroup but not in the older one (Table 2).

Adverse events Two types of side effects were noticed shortly after antibody administration but did not lead to dropouts [5]. The first consisted of mild to moderate flu-like symptoms attributed to cytokine release that rapidly disappeared under medication. The second was a transient EBV reactivation that was first observed on day 7 following

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p values express statistical significance of the difference between the number of patients in the placebo and ChAglyCD3 subgroups at month 0 or month 48

the first ChAglyCD3 injection; EBV DNA copies peaked between days 21 to 28 and returned to normal pre-treatment levels between days 35 and 45. During this period of reactivation one patient presented a cervical adenopathy and fever for 12 days; in 15 other patients symptoms were mild and resolved within a few days (sore throat, $n=15$; one to three enlarged cervical lymph nodes, $n=7$; fever, $n=4$). Over the 48 month follow-up period, no biological or clinical signs of EBV reactivation or EBV-related disease were observed; there was no higher incidence of infections, and no lymphoma or other types of cancer. No differences in CD3+ lymphocyte counts were found between months 6 and 48 (Fig. 3). The ChAglyCD3 group showed a decreased CD4+/CD8+ ratio at months 6 and 12 ($p<0.001$), which appeared mainly to be the result of a slightly higher CD8+ lymphocyte count ($p<0.05$); CD4+/CD8+ ratio and CD8+ counts were normalised at later time points.

The ChAglyCD3 group did not have the same weight gain and increase in BMI as the placebo group during the first 24 months, but then proceeded at the same rate. Over 48 months, the mean body weight for the ChAglyCD3 group increased by 4.4 kg (BMI +1.4 kg/m²) vs +6.6 kg (BMI+2.1 kg/m²) in the placebo group (ESM Fig. 2).

Discussion

The present study shows that a short treatment with a CD3 antibody suppresses the rise in insulin requirements that occurs in type 1 diabetic patients during the first few years after diagnosis. This effect is correlated with a young age at onset and with higher initial residual beta cell function as independent determinants. It was associated with a prevention or reduction of further losses in beta cell mass, an effect that waned with time. In the younger subpopulation, the antibody treatment was associated with better metabolic control variables during the 48 month follow-up period.

In our placebo group, aged 12 to 39 years, mean insulin dose increased progressively from the start of the study; this can be attributed to a continued decrease in the residual beta cell function, probably the result of further beta cell losses caused by the autoimmune process or/and by the chronic hyperglycaemic state [16, 17]. The ChAglyCD3-treated group did not have a decrease in function during the first 24 months, suggesting that the antibody had suppressed the beta cell destructive process. This beneficial effect waned in time as indicated by the decline in beta cell function and the rise in insulin requirements from month 24 onwards. It is conceivable that residual beta cells are lost because the autoimmune reactivity had only been transiently suppressed; their survival and/or function may also have been affected by the chronic state of hyperglycaemia. Since the beta cell mass is already markedly reduced at clinical onset of type 1 diabetes [18, 19], it is likely that any immune-modulating intervention will have to be associated with, or followed by, a beta cell replacement therapy, as recently tested in rodents [20]; a combination with other methods for tolerance-induction should also be considered [21, 22].

Multivariate analysis indicated that the baseline residual beta cell function as well as age are two baseline variables that are independently correlated with the effect of ChAglyCD3 on insulin requirements over 48 months. The influence of the initial beta cell function has already been observed in our 18 month analysis [5]. We now show that the antibody effect is maintained throughout the 48 month follow-up in the subpopulation with initial beta cell function ≥ 50 th percentile, while being absent in the other patients. The influence of age was also illustrated by a post hoc analysis of antibody effects in patients aged above or below the 50th percentile of the study population age, i.e. 27 years. In the younger subpopulation, aged 12–26 years, antibody treatment kept insulin requirements under baseline values for at least 3 years, which can be explained by the preservation of the initial beta cell function; in the placebo group beta cell function rapidly declined and insulin requirements increased markedly during this period. Our study is underpowered to determine whether the age effect

Fig. 3 Lymphocyte subsets in the two patient groups. Circles represent the placebo group and triangles the ChAglyCD3 group. All values are means $\pm$ SE. For each value the number of patients is listed for whom data were available at that time point. Statistical difference between groups: * $p<0.05$, *** $p<0.001$

is linear. In the older subpopulation, aged 27–39 years, no preservation of residual beta cell function was seen in the antibody-treated patients; their transient decrease in mean insulin dose may have been partly caused by some insulin under-treatment as suggested by their mean HbA_{1c} levels, which are higher than in the placebo subgroup, although not statistically significant.

An influence of age was not detected in the trial conducted by Herold et al. This might be related to the different age range (7.5–30 years) or distribution in their study, or to its shorter follow-up [4, 6]. The present finding that the effects of ChAglyCD3 are much more pronounced in younger patients can be explained by an age-dependency of the insulinitis process. In an analysis of autopsy reports at or shortly after clinical onset, we noticed that insulinitis was detected in all children, but in fewer adolescents and rarely in adults [19, 23]. In clinical terms, older patients with type 1 diabetes are also presumed to undergo a less aggressive form of the disease [24, 25]. Consistent with this view is the more rapid and pronounced loss of residual beta cell function that we observed in our younger placebo subgroup compared with the older placebo subgroup.

In the younger patients, the antibody-induced preservation of beta cell function was associated with better glucose control variables. This is consistent with the DCCT study in which patients with higher beta cell function had a better glucose control and those with a loss of residual beta cell function an increased risk for severe hypoglycaemia [26, 27]. In the young placebo subgroup, virtually all patients progressed within 4 years to a metabolic state with wide variability in FBG and with HbA_{1c} levels >6.5%, despite increased insulin doses. On the other hand, one-half of the antibody-treated patients maintained a relatively stable and normal FBG and HbA_{1c} levels <6.5% with daily insulin doses <0.5 U/kg body weight. The antibody-induced lowering of HbA_{1c} levels was also statistically significant when mean values were compared with those after placebo. In the study by Herold et al. an antibody-effect on HbA_{1c} levels was reported for the first 18 months [6]. A reduction in glucose fluctuations and a lowering of HbA_{1c} levels are clinically relevant variables as they predispose to decreased risks for hypoglycaemic events [28] and chronic diabetes lesions, respectively [29]. The latter effects could not be evaluated in the present study. During the first 4 years after onset, the number of hypoglycaemic events was low and signs of chronic lesions remained absent; moreover, the size of the subgroups would not provide the power to detect differences and to relate them to a preservation of residual beta cell function. Our observations also suggest that the improvement in metabolic variables might be limited to the first 4 years. They do, however, demonstrate that a short immune intervention can prevent losses in residual beta cell function and in metabolic control variables for at least

3 years, which supports the potential of intervention strategies at or before clinical onset of type 1 diabetes.

The prolonged effect of a short immune treatment is, to our knowledge, novel in the field of autoimmune disease. It is often assumed that an autoimmune disease will need continuous treatment to maintain efficacy; this would then increase risk of infection, as seen with TNF antagonists [30, 31] or with natalizumab [32, 33], or of nephropathy with ciclosporine [34]. In our limited follow-up period, none of the patients developed cancer or lymphoproliferative disease, or presented abnormal or more frequent infectious episodes. There were, however, side effects during and shortly after antibody administration as result of cytokine release and of EBV reactivation; although transient, their nature stresses the need to seek doses and regimens that avoid or minimise these adverse events. This will be necessary before extending anti-CD3 trials to target populations that seem most promising according to the present findings. Preservation of residual beta cell function was indeed best achieved and maintained in the younger population and in patients in an apparently early phase of the destruction process; forthcoming trials should therefore preferentially recruit young patients, including children. A safe regimen that suppresses progression of the disease at clinical onset will also stimulate trials in individuals at high risk for developing type 1 diabetes.

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Appendix

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