

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Keymeulen B, Vandemeulebroucke E, Ziegler AG, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med* 2005;352:2598-608.

Appendix 1: Screening, Randomization and Follow-up Visits

PATIENT SCREENING AND SELECTION:

Between 07/06/2000 and 07/03/2003, 210 recent onset Type 1 diabetic patients were consecutively screened by 56 diabetologists that are members of the Belgian Diabetes Registry (BDR) and by 17 diabetologists in Germany. Blood samples were taken and sent to the BDR core facility for determination of plasma glucose, C-peptide, ICA and GADA. A file with clinical information was added. When inclusion-criteria described in methods were met, the patient was informed about the protocol and, upon written informed consent, transferred to one of the 5 trial centers, 4 in Belgian universities (UZ Gasthuisberg Leuven, Academisch Ziekenhuis VUB, Hôpital Erasme ULB, UZ Antwerpen) and 1 associated to Munich university (Hospital München-Schwabing).

Of 210 newly diagnosed patients that were screened between June 2000 and March 2003, 39 refused to participate and 91 did not meet the inclusion criteria (44 with random C-peptide < 0.20 nmol/l, 36 were ICA+GADA negative, 11 for other parameters). The remaining 80 patients were all randomized, 40 to the ChAglyCD3 group and 40 to the placebo group.

STUDY DRUG:

ChAglyCD3 or placebo preparations were produced in the Therapeutic Antibody Center in Oxford. Eighty boxes, containing each ten vials, were sent to the BDR core facility and stored at -80 °C. Each box was coded and this code was labelled on each vial in this box. There were no apparent macroscopic differences between vials irrespective of the box.

RANDOMIZATION:

On admission of the patient, the BDR core facility was contacted by telephone to start the randomization process. A member of the BDR core facility contacted then by telephone Dr Bart Van der Auwera (“third party”: member of the Diabetes Research Center Brussels Free University-

VUB- and not working at the BDR core facility), who assigned each patient to a coded treatment box. This assignment was done according to guidelines that were defined by the statistician of the project (Prof. L. Kaufman). Randomization was performed using a centralized minimization procedure balancing for center (n=5), age (<15yrs vs. ≥15 yrs), and ICA positivity. The same weight was given to each of the 3 factors. Dr Bart Van der Auwera had received from the Sir William Dunn School of Pathology, Oxford, England a list with 40 coded numbers, referring to ChAglyCD3 and 40 other coded numbers referring to placebo treatment.

After assignment, the coded box was immediately transferred from the BDR core facility to the trial center in Belgium where the patient was hospitalized. For Germany, 27 boxes were transferred from the BDR core facility to the trial center in Munich and stored at -80°C. After assignment, the coded box was taken out from their freezer.

Until today all patients, all personnel at the trial center, and at the BDR core facility and all laboratory workers are unaware of treatment assignment as none of the codes had to be broken for medical reasons. However, as ChAglyCD3 treatment can be associated with a flu-like syndrome, patients and workers at the trial center could, in principle, have guessed their treatment assignment. It is important to emphasize that this infringement of the double-blind condition of the trial did not apply to the efficacy parameters including in particular measurement of C-peptide. In fact, all the samples for these studies were centralized by the BDR core facility that performed the analysis and the results, which were then introduced in the central database, were not communicated to the clinician. In all cases, particular attention was devoted to the fact that patients should remain blinded during the study.

FOLLOW-UP:

After treatment during hospitalization, patients visited the trial centers at weeks 2, 3, 4, and 6 and at month 3, 6, 9, 12, 15, and 18.

Appendix 2. Sample Size Calculation, Definition of Primary Outcome, Time Log of Statistical Analysis and Related Information

The sample size calculation of the original protocol was based upon the detection of a difference of 40 percent between the two groups in the frequency of becoming C-peptide negative ($\leq 0.3\mu\text{g/L}$) at 12 months post-treatment. With data for 34 evaluable patients in each treatment group, there is 90 percent power of detecting such a difference (60 percent in the placebo group and 20 percent in the ChAglyCD3 group), by means of a t-test performed two-sided at the 5 percent level of significance. To account for a drop-out rate of 15 percent, 80 patients were to be enrolled in the study.

After the first meeting with the DSMB in June 2002 and taking in to account data in the literature about the low frequency of C-peptide negativity in adults at one year after diagnosis of type 1 diabetes, it was decided to select as primary efficacy variable, the change in mean C-peptide during the hyperglycaemic phase of the clamp (60 to 140 min.) in absence of glucagon, and this between start and month 6. This decision on the primary variable was made before unblinding the data, and the protocol was amended accordingly. Beta-cell function during the hyperglycaemic phase of the clamp (60 to 140 min) in the absence of glucagon is expressed in this article as AUC of C-peptide during this hyperglycaemic phase (60-140 min) in the absence of glucagon, as suggested by both reviewers.

After all efficacy data for all patients concerning the first 6 months after treatment became available, the treatment was unblinded. This was done in 2 phases. First the 2 groups were identified as A and B and descriptive statistics were calculated for all primary and secondary efficacy variables (23 December 2003). After review of these descriptive statistics by the clinical

coordinator and the principal investigator, A and B were identified and the analysis of these data was completed (10 February 2004).

The data of the visits between 6 and 12 months were analyzed after all patients had completed this period (13 May 2004). The data of the visits between 12 and 18 months were analysed after all patients had completed this period (15 September 2004).

All statistical analyses were performed in accordance with a prespecified statistical protocol. Multiple regression analysis was used to evaluate the effect of potentially prognostic factors on the change of glucose-clamp-induced C-peptide release (AUC/min between 60 to 140 minutes) between the start of the study and month 18. The predefined prognostic factors were age, gender, protective versus non-protective genotype, glucose-clamp-induced C-peptide release, HbA_{1c}, body weight, BMI, and islet-cell and glutamic acid decarboxylase antibody levels at start of treatment. Separate analyses were performed for the ChAglyCD3 and placebo treatment groups; an analysis was then conducted on glucose-clamp-induced C-peptide release, insulin dose and HbA_{1c}, using two-way analysis of variance and the following factors: insulin dose, status of AUC of glucose-clamp-induced C-peptide release before treatment, and their interaction.

In order to examine whether the degree of residual beta-cell function at start influenced the effect of treatment on insulin dose and HbA_{1c}, ChAglyCD3 and placebo treated patients were divided in two groups depending on whether their initial C-peptide during hyperglycaemic phase of the clamp (AUC 60 to 140 min.) in absence of glucagon was lower or higher than the median that characterized the entire population at start (P50=0.518 nmol/l/min).

Appendix 3. Demographic and Immune Markers for the <P50 and ≥P50 Subgroups

	< P50		≥P50	
	Placebo	CD3	Placebo	CD3
n	16	24	24	16
Gender (Male/ Female)	11/5	17/7	16/8	8/8
Age (years)	26 ± 7	27 ± 7	26± 8	28 ± 7
At Diagnosis				
Diabetic ketoacidosis (%)	1/14 (7%)	5/17 (29%)	4/15 (27%)	3/11 (27%)
Polyuria and weight loss (%)	10/14 (71%)	18/21 (86%)	9/23 (39%)	7/13 (54%)
Weeks since onset of symptoms*	10 (3-15)	4 (3-19)	2 (2-4)	4 (4-14)
At screening				
Days since diagnosis*	6 (5-10)	7 (3-14)	11 (6-17)	14 (7-18)
% patients positive for ICA	69	79	83	75
GADA	88	88	83	94
At start of treatment				
Days since diagnosis*	23 (17 - 26)	20 (16 - 24)	25 (21 - 27)	22 (18 - 24)
Days of insulin treatment*	19 (13-25)	20 (16-24)	21 (15 - 25)	19 (18-23)
Body weight (kg)	61 ± 10	67 ± 13	68 ± 14	68 ± 18
BMI (kg/m ²) *	20.8 (18.8-22.1)	21.8 (20.3-23.3)	21.3 (19.5-23.5)	21.9 (20.1-24.1)
Insulin dose (U/kg/day)	0.45 ± 0.25	0.39 ± 0.21	0.48 ± 0.28	0.36 ± 0.19

Data are presented as mean ± SD (in case of normal distribution) or as median (quartile range)* (in case of significant deviation from a normal distribution).

ICA, GADA denotes anti-islet cell, glutamic acid decarboxylase autoantibodies respectively.

Appendix 4. Body temperature (°C) following ChAglyCD3 administration (48mg cumulative dose; 6 consecutive doses of 8mg)

ChAglyCD3	Median	IQR1	IQR3	Min	Max
1st dose	38,4	38,2	38,7	37,3	39,9
2nd dose	38,2	37,8	38,6	36,8	39,4
3rd dose	38,0	37,8	38,3	36,8	39,5
4th dose	37,4	37,0	37,7	36,2	38,1
5th dose	36,9	36,8	37,3	36,0	38,9
6th dose	36,9	36,7	37,1	35,9	37,9