Title Page

CLINICAL STUDY REPORT

Study Title:	The therapy of type 1 diabetes with *ex-vivo* expanded CD4+CD25+CD127- T regulatory cells (Tregs) and anti-CD20 monoclonal antibody – the randomized trial
Short title:	Treg Vac 2.0
Protocol Number:	NKBBN/374/2012-NKBBN/374- 7/2014, version 09_2-14, dated 30-Sep-2014
Study Phase:	I/II
Study design:	Prospective, multicenter, randomized [1:1], open-label (regarding Tregs treatment and the control group), single-blinded (patient-blinded regarding anti-CD20 antibody rituximab treatment), placebo-controlled, parallel-group study
Compound:	T-reg cells preparation/rituximab
Indication:	Type 1 diabetes
Study Sponsor:	Medical University of Gdańsk, Skłodowskiej-Curie 3a, 80-210 Gdańsk Poland
Coordinating Investigator:	Prof. dr hab. Piotr Trzonkowski, Katedra I Zakład Immunologii Medycznej Gdański Uniwersytet Medyczny ul. Dębinki 7, budynek 27, IIp. 80-952 Gdańsk tel. 58 3491590, 58 3491593 fax 58 3491591 e-mail: ptrzon@gumed.edu.pl; p.trzonkowski@poltreg.tech
Study Initiation Date:	15-Apr-2015
Study Completion Date:	31-Dec-2019
EudraCT Number:	2014-004319-35
Report Date:	**Document Version** / **Date** **1.0** / 18-Nov-2020

Synopsis

Study Title: The therapy of type 1 diabetes with *ex-vivo* expanded CD4+CD25+CD127- T regulatory cells (Tregs) and anti-CD20 monoclonal antibody - the randomized study

Study number: EudraCT 2014-004319-35

Study phase: Phase ½

Name of Study Intervention: Treg cells preparation and rituximab

Name of Sponsor/Company: Medical University of Gdańsk, Skłodowskiej-Curie 3a, 80-210 Gdańsk

Number of study center(s) and countries: This study was conducted in 45 patients randomized at **4** centers that enrolled patients in **Poland.**

Study period (years):

- **Date of first enrollment:** 02-June-2015

Date of last completed: 18-Oct-2019 (last patient last visit)

Rationale: Type 1 diabetes is a condition in which pancreatic islets are destroyed by self-reactive effector T cells. The process is facilitated by deficits in the number and suppressive activity of T regulatory cells (Tregs). The administration of expanded autologous CD4+CD25+FoxP3+ Tregs in children and adolescents with recently diagnosed type 1 diabetes can delay the process of pancreatic islets destruction by suppressing self-reactive effector T cells. Early administration and repetitive doses of Tregs seem to positively affect this suppressive effect.

B lymphocytes seem also contribute to the pathogenesis of the disease as their selective depletion with the anti-CD20 antibody rituximab results in preserved beta-cell function in patients with type 1 diabetes of recent onset. Although clinically significant, the therapeutic effect of these two therapies is only transient, and patients eventually develop diabetes.

In this study, these two therapeutic approaches were combined. Patients were recruited in earlier phases of the disease and treated by repetitive administration of Tregs and anti-CD20 antibody rituximab with the aim of significantly prolonging insulin independence and further delaying disease onset.

Listed below are the objectives and endpoints that are described in this report.

Study Objectives	
Objectives	**Endpoints**
Primary	
To assess the safety and efficacy of the treatment in individual patient groups treated with Tregs or the combination of Tregs and anti-CD20 antibody rituximab	C-peptide level (fasted/post MMTT stimulation and after glucagon test) at 2 years after first dose of Tregs Daily insulin dose per kg of body weight (DDI) 2 years after the first dose of Tregs Number of treated patients in remission 1 and 2 years after first dose of Tregs – the number of patients with DDI lower than 0.5U/kg/day and HbA1c lower than 6.5% Number of adverse events (AEs) reported 2 years (week 104) after the first dose of Tregs

Secondary	
To assess hypersensitivity reactions and immunosuppressive side effects of Tregs or Tregs in combination with anti-CD20 antibody rituximab	Assessment of the occurrence and severity of side effects directly related to Tregs (hypersensitivity reactions, injection-site thromboembolic events) and blood sampling (>2 g/dL drop in hemoglobin levels) Assessment of the occurrence and severity of effects directly related to anti-CD20 antibody rituximab administration (hypersensitivity reactions) Assessment of the occurrence and severity of side effects associated with administration of Tregs or anti-CD20 rituximab antibodies, primarily immunosuppressive effects: occurrence of infections of any etiology and *de novo* tumors detected Any serious AE (SAE) in two or more patients with confirmed association to the administration of therapy These four secondary safety endpoints will be documented as AEs of special interest (AESI) and related treatment-emergent AEs (TEAEs), where appropriate
To investigate further efficacy and side effect parameters for the investigational medicinal product (IMP) given	C-peptide level (fasted) (weeks 2, 5, 12, 26, 39, 52, 65, 78, 92, and 104) C-peptide level (post-MMTT stimulation and glucagon test) (weeks 12, 26, 52, 78, and 104) Exogenous insulin dose per kg of body weight (weeks 2, 3, 4, 5, 12, 14, 26, 39, 52, 65, 78, 92, and 104) The proportion of insulin-independent patients (DDI = 0 UI/kg body weight [b.w.]) (weeks 52 and 104) The proportion of patients in remission (DDI ≤ 0.5 UI/kg b.w. and HbA1c lower than 6.5%) (weeks 52 and 104) HbA1c level (%) (week 2, 5, 12, 26, 39, 52, 65, 78, 92, and 104) as glycemic control (fasting average of 7 days) The amount and intensity of side effects of therapy (weeks 52 and 104) Peripheral blood lymphocyte immunophenotype (weeks 2, 5, 12, 14, 26, 39, 52, 65, 78, 92, and 104) with basic phenotype results

Statistical methods:

The statistical analysis was performed by ICRC-Weyer GmbH, Berlin.

The statistical analysis plan (SAP) is based on the trial protocol TregVac 2.0 (final version 09_2-14, dated 30-Sep-2014, and is detailed in the Final SAP (SAP v2.0).

Demographic safety, and efficacy data are described by patient and in summary tables by treatment with graphics.

To perform comparisons of efficacy between treatment groups (Tregs+anti-CD20 antibody rituximab/Control, Tregs/Control, and Tregs/Tregs+anti-CD20 antibody rituximab), analysis of covariance (ANCOVA) was performed on logarithmized values for the following parameters for baseline to the 24-month visit and for all visits: AUC of C-peptide levels (MMTT, 0-240 min), AUC of C-peptide levels (glucagon test, 0 to 6 min), C-peptide concentration (glucagon test, 0 to 6 minutes), C-peptide levels (MMTT, 0-240 min), C-peptide levels (fasted). These values were back-transformed to the normal scale to obtain geometric mean ratios and their 90% confidence limits. For DDI, an ANCOVA based on the original values was performed to obtain DDI differences and the 90% confidence interval.

Repeated Measures Analysis of Covariance (RMANCOVA) was performed for all follow-up visits (starting from month 3) to analyze comparisons of geometric mean ratios between treatment groups and their 90% confidence intervals by the following parameters: AUC of C-

peptide (MMTT), AUC of C-peptide and C-peptide concentration (glucagon test, 0 to 6 min), C-peptide levels (MMTT, 0-240 min), C-peptide levels (fasted), HbA1c values, glucose levels, and DDI.

Survival analysis was performed for remission and time to first loss of insulin independence starting from month 3. Contingency tables resulting from exact permutation test analyses with p-values are also provided for these parameters.

Safety parameters were analyzed using descriptive statistics. Quantitative data (e.g. hematology, blood chemistry, urinalysis, and vital signs) were described by the following summary statistics: arithmetic mean, standard deviation, median, minimum and maximum and presented by renal function group for the original data as well as for the difference to the respective baseline, if appropriate

Methodology:

This was a phase 1/2, prospective, randomized [1:1], open-label (regarding Tregs treatment, control group) and single-blinded (patient blinded, regarding anti-CD20 antibody rituximab treatment groups), multi-center clinical study performed in children and adolescents with type 1 diabetes. There were two parallel groups and a treatment-free control arm (total N=45, Tregs group N=15, Tregs+anti-CD20 rituximab group N=15, treatment free control group N=15).

The total duration of the study was up to 42 months, divided as follows:

- Inclusion of patients: 18 months
- Observation time (for each patient): 24 months

Patients aged 8-16 years who were diagnosed with type 1 diabetes not earlier than 2 months before the application of the first dose were included in the study,

Patients were randomized to receive doses of Tregs between 10 and $30x10^6$/kg (up to not more than $60x10^6$ cells/kg in total) at Day 0 and Day 90. Those randomized to treatment with both Tregs and anti-CD20 antibody received anti-CD20 antibody rituximab at 375 mg/m^2 at study days: 14, 22, 29 and 36.

Number of patients (planned and analyzed):

In all, 45 patients were randomized to a treatment group, and 36 of these 45 patients were screened. Of the 36 screened patients, 25 received at least one dose of any active IMP and 11 patients had been assigned to the control group. All 36 screened and randomized patients were included in the Safety Set. Patients in this population were used for safety analyses and analyzed as treated. All 36 patients had at least one assessment of the primary efficacy variable beyond the baseline assessment up to month 24 and were therefore also included in the intention-to-treat (ITT) group. Patients in this population were used for the primary analysis of efficacy and analyzed as randomized. Of these 36 patients, 35 completed the trial without any major protocol violation and had the month 24 assessment of the primary efficacy variable; these patients were included in the per-protocol (PP) set. One patient had a protocol deviation considered important (diagnosis of autoimmune-mediated multiorgan endocrine insufficiency) with regard to influence on main objectives, and this patient was excluded from the PP set.

Diagnosis and main criteria for inclusion:

Children and adolescents aged 8 to 16 years with a body mass index (BMI) the range of 25th-75th percentile according to the study "Elaboration of reference blood pressure ranges for children and adolescents in Poland", PL 0080 (OLAF); fasting plasma C-peptide level more than 0.7 ng/mL and increased by ≥ 100% in a stimulation test; and the presence of anti-islet autoantibodies. The diagnosis of T1DM had to be not more than 2 months before the trial started. Patients and their parents were required to be involved in intensive diabetes management, defined as self-monitoring of glucose values no less than three times / day and appropriate administration of insulin and to have appropriate venous access for blood drawing.

Study Interventions, Dose, Mode of Administration, and Batch Number(s):

Patients enrolled received two infusions of up to $60x10^6$ per kg b.w. Tregs at day 0 and 90 and four infusions of anti-CD20 antibody rituximab or a matching placebo at day 14, 22, 29 and 36. The list of batches of anti-CD20 antibody rituximab is provided in Appendix 16.1.6.

Duration of study intervention:

The total duration of the study was up to 42 months, divided as follows:

- Inclusion of patients: 18 months
- Observation time (for each patient): 24 months

Summary of Results and Conclusions

The results of the primary and secondary efficacy analyses strongly support the notion that both treatments delayed progression of T1DM in this patient population. Indicators of T1DM progression, including C peptide (fasted, MMTT, and glucagon test), HbA1c, glucose, and DDI, were consistently superior, often statistically significantly so, in the two treatment groups. They were also consistently superior in the combination therapy / monotherapy comparisons, with the inferiority of the monotherapy group indicated in some cases. The proportion of patients in remission was consistently better in the combination therapy group (but not the monotherapy group) than in the control group, and there was a clear trend for the time to first loss of remission to occur later in patients in the combination therapy group than in either the monotherapy group or the control group. Finally, the first loss of insulin independence also tended to occur later in the combination therapy group than in either the monotherapy group or the control group, but these differences were not significant.

No deaths, serious adverse events (SAEs), severe adverse events (AEs), or AEs leading to withdrawal of study treatment were reported during this study.

Demographic and Other Baseline Characteristics:

Patients were White Caucasian male (47.2%) and female (52.8%) patients aged 9 to 16 years old (mean 12.8±1.67 years). All patients were diagnosed with type 1 diabetes at least 2 months prior to the study (mean, 5.9±3.85 months prior to screening). The following additional demographic characteristic were recorded: weight (48.03±9.719 kg), height (159.0±9.87 cm), and BMI (18.740±1.747). The following autoantibodies were recorded at recruitment as baseline disease characteristics: blood glutamic acid decarboxylase (658.201±784.280 IU/mL), blood islet cell antibody titer (96.7±153.62), blood insulin

autoantibody (6.425±6.746 IU/mL). No differences of demographic data and baseline characteristics had been observed between the treatment groups.

Exposure:

In the 12 patients in the Tregs+anti-CD20 rituximab antibody group, all 12 patients received two Tregs doses, and all patients received 4 rituximab doses. In the 13 patients in the Tregs+placebo group, the mean number of Tregs doses was 1.9±0.28 doses (1 patient did not receive 1 dose), and the mean number of rituximab doses was 3.8±083 doses (1 patient received only 1 dose).

Efficacy Results:

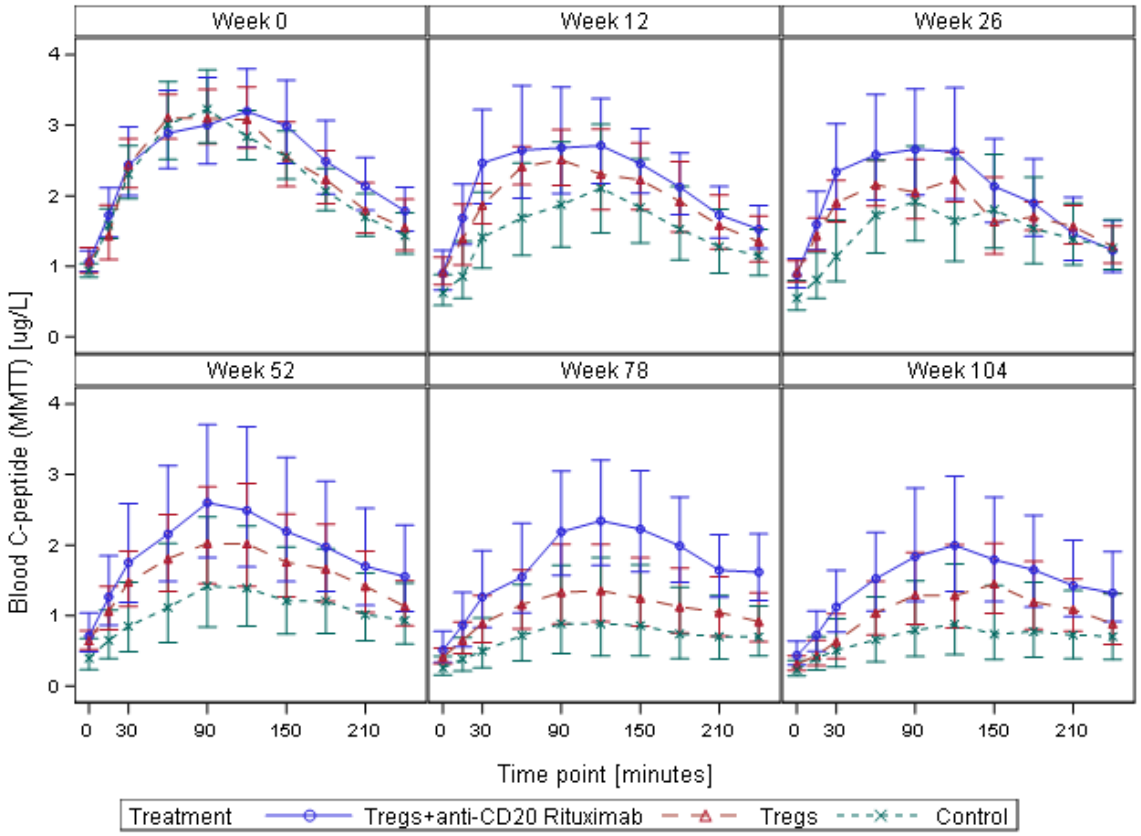
This study investigated the safety and efficacy of a cell therapy including administration of isolated and expanded CD4 + CD25 + T regulatory cells (Tregs) with or without the anti-CD20 antibody rituximab in male and female patients aged 9 to 16 years who had been diagnosed with type 1 diabetes mellitus (T1DM) at least 2 months prior to enrollment. All 36 enrolled patients were included in the intention-to-treat (ITT) set (13 in the Tregs+anti-CD20 rituximab group, 12 in the Tregs group, and 11 in the control group).

C peptide levels

C peptide levels are routinely used to assess the function of the pancreas in diabetic patients as it is produced in parallel with insulin but degrades more slowly. Most children diagnosed with T1DM will become insulin-deficient within 2-3 years of onset, and C peptide levels decrease in parallel with insulin levels. The point estimates for the ratios Tregs+anti-CD20 rituximab/control and Tregs/control showed that at the 24-month analysis, in the MMTT test, both treatment groups (1.770, 90%CI 1.018-3.078 and 1.893 90%CI 1.062-3.372 in the Tregs+anti-CD20 rituximab and Tregs groups, respectively) had statistically significantly higher AUC of C peptide values than the control group, whereas in the analysis of all visits, only the combination therapy improved AUC of C peptide (at 12 [1.666 90% CI 1.069-2.594], 18 [2.170 90% CI 1.2573.744], and 24 [1.955 90% 1.058-3.613] months). C peptide levels were also analyzed following the MMTT at 24 months and all visits. At 24 months, both treatment groups had statistically significantly superior C peptide values compared the control group at one timepoint (150 min) (1.877 90%CI 1.019-3.456 and 2.204 90%CI 1.181-4.111). However, over all visits, values were statistically significantly superior to the control group at 50% of the timepoints in the Tregs+anti-CD20 antibody rituximab group but only 3 (6%) of the timepoints in the Tregs group.

While there was no difference between the two treatment groups at any timepoint in the AUC of C peptide and C peptide level (MMTT) analysis; geometric mean time plots showed that while AUC of C peptide values and C peptide levels decreased over time in all 3 groups, they remained consistently highest in the combination therapy group. This suggests that although both treatments benefited patients, the combination therapy may be better at slowing the loss of insulin production in this population.

Figure 1-1: Geometric Mean Concentration Time Plots of C-peptide Levels (MMTT, 0-240 min) (ITT Set)



Abbreviations: Tregs: T regulatory cells, MMTT: mixed meal tolerance test, ITT: intention-to-treat, min: minutes
Note(s): Geometric means and their 90 % confidence intervals are presented.
Source: Figure 14.2.1.3.7

AUC of C peptide and C peptide levels were also measured in the glucagon test (0 to 6 min) at 24 months and all visits. While neither treatment group performed consistently better than the control group, the monotherapy was statistically significantly inferior to the combination therapy (0.533, 90% CI 0.305-0.932) in the analysis of AUC of C peptide treatment geometric mean ratio in the 24-month visit analysis. C peptide levels in the glucagon test were statistically significantly higher in the combination therapy group than the control group at the 0-minute timepoint at 6 months (1.532, 90% CI 1.022-2.295), 18 months (2.591, 90% CI 1.615-4.154), and 24 months (1.717, 90% CI 1.019-2.896) and at the 6-minute timepoint at 12 months (1.738, 90% CI 1.012-2.986) and 18 months (2.431, 90% CI 1.371-4.309) but at only one visit (18 months; [1.630, 90% CI 1.026-2.590]) in the Tregs group.

When AUC of fasted C peptide levels were evaluated at 24 months, the point estimates of the treatment geometric mean ratio showed that only the combined therapy was superior to the control (2.268, 90%CI 1.264-4.069); furthermore, the comparison of the two treatment groups showed the monotherapy was inferior to the combination therapy (0.553, 90%CI 0.390-0.989). When analyzed at all visits, point estimates of treatment geometric mean ratios for AUC of C peptide levels were higher in both treatment groups towards the control group at 3 months (1.729, 90% CI 1.229-2.433 and 1.811, 1.301-2.523, respectively), 6 months (2.638,

90% CI 1.81-3.84 and 2.727, 90% CI 1.897-3.920, respectively), 12 months (2.618, 90% CI 1.640-4.177 and 2.437, 90% CI 1.548-3.834, respectively), 15 months (2.478, 90% CI 1.539-3.989 and 1.773, 90% CI 1.111-2.830, respectively), and 18 months (3.901, 90% CI 2.508-6.066 and 2.457, 90% CI 1.597-3.781, respectively), but only the combination therapy was superior to the control group at 21 and 24 months, and the monotherapy was inferior to the combination therapy at 18 (0.630, 90% CI 0.416-0.955), 21 (2.919, 90% CI 1.70-5.00), and 24 (0.528 90% CI 0.314-0.889) months.

Other indicators of diabetes progression

Hemoglobin A1c (HbA1c) can be used to predict the progression of T1DM in children, with higher levels correlated to more advanced disease. Point estimates for treatment geometric mean ratios of HbA1c were significantly lower in both treatment groups than in the control group at 3 months, but only the combination therapy group had significantly lower levels than the control group at later timepoints (3, 6, 9, 12, 21, and 24 months). Importantly, the combination therapy was superior to the monotherapy at every visit except for the 9-month visit. These findings are strong support for the notion that the combination therapy may slow disease progression in children.

Glucose levels are a marker of progression in T1DM, with higher levels indicating more advanced disease. Point estimates for treatment geometric mean ratios of glucose showed that there was no statistically significant difference in glucose levels between the control group and either treatment group. However, most comparisons between the two treatment groups showed non-inferiority of the monotherapy, especially in year 2 (months 3, 15, 18, 21, and 24).

Insulin use and Loss of insulin dependence

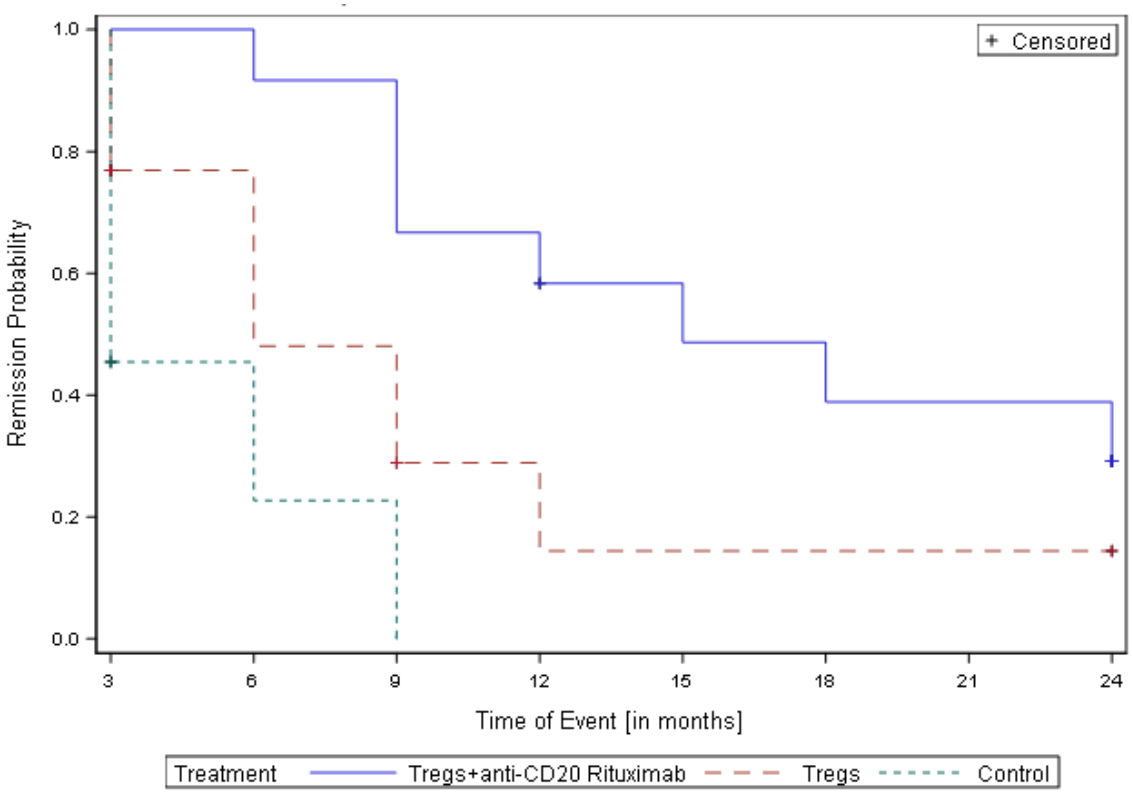
Mean DDI increased during the study in all groups. Point estimates for the treatment comparisons showed that neither treatment significantly affected DDI at 24 months. However, when analyzed over all visits for the treatment group differences, the Tregs+anti-CD20 antibody rituximab group was statistically significantly superior to the control group (90% CI completely below 0) at month 6 (-0.144, 90% CI -0.276 - -0.012), month 12 (-0.233, 90% CI -0.386 - -0.079), month 15 (-0.243, 90% CI -0.385 - -0.100), month 18 (-0.205, 90% CI -0.376 - -0.035), month 21 (-0.287, 90% CI -0.460 - -0.114), and month 24 (-0.173, 90% CI -0.345 - -0.002), while the Tregs group was statistically significantly better than the control group only at month 15 (-0.163, 90% CI -0.309 - -0.016) and month 21 (-0.287, 90% CI -0.441 - -0.082). While comparison of the two treatments revealed they were not statistically significantly different at any timepoint, a geometric mean concentration time plot of DDI showed that DDI was consistently lowest in the combination therapy group (although at most timepoints its standard deviations overlapped those of the monotherapy group).

Remission was analyzed with the exact permutation test towards an alpha level of 5%. When analyzed over the course of the study, the proportion of patients in remission was significantly higher in the Tregs+anti-CD20 antibody rituximab group than in the control group at 3 (p=0.0017), 6 p=0.0029), 9 (p=0.0194), and 21 (p=0.0421) months but not at 18 (p=0.0626) or 24 (p=0.2333) months. There was no significant difference between the Tregs group and the control group at any timepoint, and the proportion of patients in remission was significantly higher in the Tregs+anti-CD20 antibody rituximab group than in the Tregs group

at 6 months (p=0.0101). Additionally, there was a clear trend for the proportion of patients in remission to decrease more slowly in the combination therapy group than in either the monotherapy group or the control group. These results suggest that the combination therapy may be better in keeping patients in this population in remission.

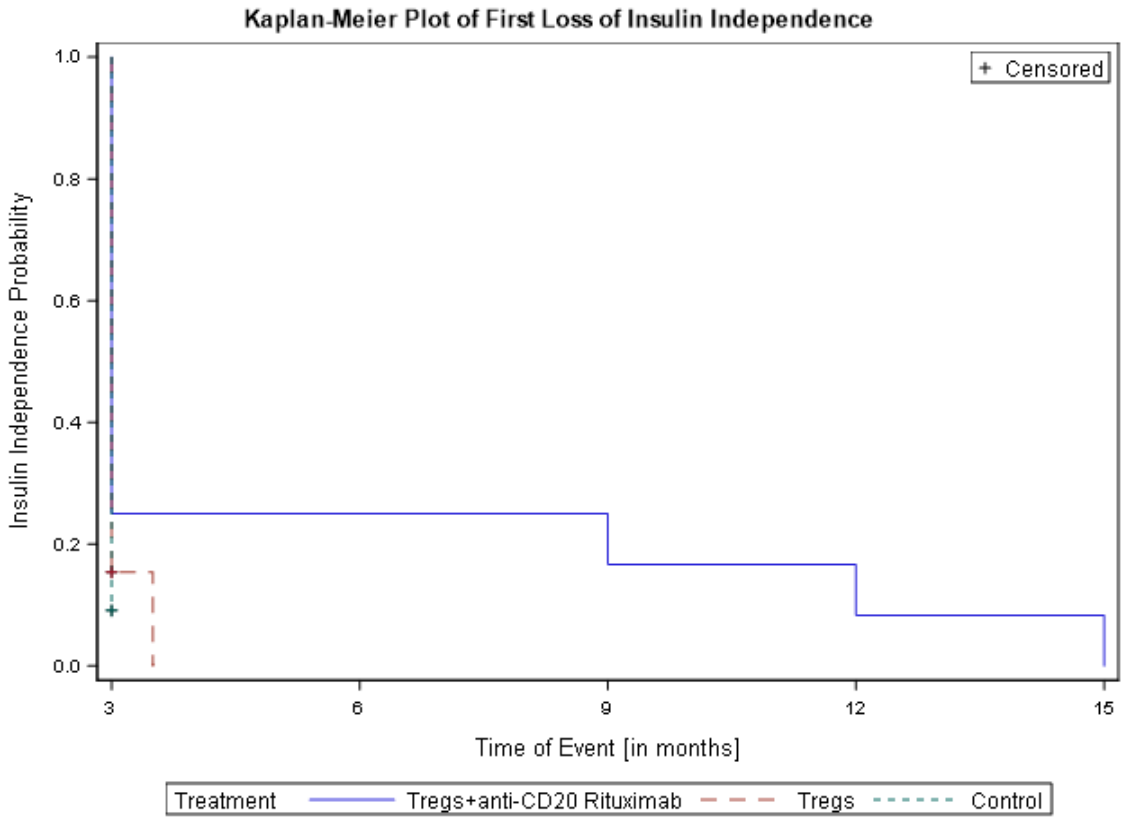
Insulin independence (DDI = 0 U/kg/day) was evaluated at the month 3-24 visits. The only significant difference among the three groups in the proportion of patients with insulin-independent status was at month 6, when there was a statistically significant difference between the Tregs+anti-CD20 antibody rituximab group and the Tregs group (p=0.0308). However, a survival analysis of time-to-first loss of insulin independence showed that first loss tended to occur later in the Tregs+anti-CD20 rituximab group than in either the Tregs or control group.

Figure 1-2: Kaplan – Meier-Plot of Time to First Loss of Remission (ITT Set)



Abbreviations: Tregs: T regulatory cells, ITT: intention-to-treat
This figure shows the results of a survival analysis with the time to first loss of remission status at the month 3 visit as the starting point. Visits 3m, 6m, 9m, 12m, 15m, 18m, 21m and 24m are included in the analysis.
If the remission status of a subject is not known for a scheduled visit, the subject will be censored from that visit onward
Source: Figure 14.2.1.6.7

Figure 1-3: Kaplan-Meier-Plot of First Loss of Insulin Independence (DDI = 0 U/kg/day) (ITT Set)



Abbreviations: Tregs: T regulatory cells, ITT: intention-to-treat
This table shows the results of a survival analysis with insulin independency status at the month 3 visit as the starting point.
Visits 3m, 3m prim, 9m, 12m, and 15m are included in the analysis.
The control group did not have month 3 prim as a scheduled visit, whereas the active treatment groups did.
If the insulin independency status of a patient was not known for a scheduled visit, the patient was censored from that visit onward.
Source: Figure 14.2.2.9.3.2

Safety Results:

No deaths, serious adverse events (SAEs), severe adverse events (AEs), or AEs leading to withdrawal of study treatment were reported during this study.

Overall, AEs causally related to treatment were reported in 20 of 25 patients (80.0%) , including 59 events in 12 (100%) patients in the Tregs+anti-CD20 antibody rituximab group and 10 events in 8 (62.5%) patients in the Tregs+placebo group experienced at least 1 AE considered treatment-related. Adverse events of special interest (AESIs) were reported in 9 (75%) of the Tregs+anti-CD20 antibody rituximab patients and 3 (23.1%) of the Tregs+placebo patients.

One AESI, infection (27 events in 12 patients [48%] in the active treatment groups), was defined as related to the administration of the rituximab antibody (antiCD20) or the immunosuppressive activity of Tregs. AEs considered related to administration of rituximab antibody included nausea (4 events in 3 patients [25%]), headache (3 events in 2 patients [16.7%]), asthenia (2 events in 2 patients [16.7%]), vomiting (3 events in 2 patients [16.7%]),

neutropenia (2 events in 2 patients [16.7%]), and diffuse alopecia (2 events in 1 patient [8.3%]). Chills and vessel puncture site haematoma were defined as AESIs related to blood collection and administration of Tregs (1 event in 1 patient [4.0%] and 2 events in 2 patients [8.0%], respectively).

Overall conclusions

- No deaths, serious adverse events (SAEs), severe adverse events (AEs), or AEs leading to withdrawal of study treatment were reported during this study.
- While most of the patients in the treatment groups experienced AEs considered causally related to the administration of Tregs or rituximab, most AEs were mild and none were severe, suggesting that the treatments were well-tolerated in White male and female children and adolescents aged 9-16 with onset of T1DM at least 2 months prior to treatment.
- Point estimates for the ratios Tregs+anti-CD20 antibody rituximab/control and Tregs/control show that AUC of C peptide and C peptide levels (MMTT) were higher in both treatment groups than in the control group.
- For AUC of C peptide at 24 months (glucagon test), the monotherapy was statistically significantly inferior to the combination therapy (0.533, 90% CI 0.305-0.932).
- For C peptide levels (fasted) at the 24-month visit, the point estimates for treatment ratios pointed toward the monotherapy being inferior to the combined therapy (0.553, 90% CI 0.309-0.989), whereas when analyzed at all visits, the monotherapy was statistically significantly inferior to the combination therapy at 18 (0.630, 90% CI 0.416-0.955), 21 (0.511 90% CI 0.304-0.859), and 24 (0.528 90% CI 0.314-0.889) months.
- Point estimates for HbA1c were significantly lower for the combination therapy than the control at 3, 6, 9, 12, 21, and 24 months. Importantly, the combination therapy was superior to the monotherapy at every visit except for the 9-month visit.
- Most comparisons in glucose levels between the two treatment groups showed non-inferiority of the monotherapy, especially in year 2.
- Point estimates in comparison of the two treatments for DDI revealed they were not statistically significantly different at any timepoint, but geometric mean concentration time plots of DDI showed that DDI was consistently lowest in the combination therapy group
- When analyzed over the course of the study, the proportion of patients in remission was significantly higher in the Tregs+anti-CD20 antibody rituximab group than in the control group at 3 (p=0.0017), 6 p=0.0029), 9 (p=0.0194), and 21 (p=0.0421) months but not at 18 (p=0.0626) or 24 (p=0.2333) months, but there was never a significant difference between the monotherapy and the control group. There was a clear trend in the time-to-event (time to first loss of remission) analysis for the proportion of patients in remission to decrease more slowly in the combination therapy group than in either the monotherapy group or the control group.
- The proportion of patients with insulin-independent status was significantly higher for the combination therapy than for the monotherapy at month 6 (p=0.0308), and a survival analysis of time-to-first loss of insulin independence showed that first loss tended to occur later in the Tregs+anti-CD20 rituximab group than in both the Tregs and Control group.

SIGNATURE PAGE

Study title: The therapy of type 1 diabetes with *ex-vivo* expanded CD4+CD25+CD127- T regulatory cells (Tregs) and anti-CD20 monoclonal antibody - the randomized study

Trial protocol: NKBBN/374/2012-NKBBN/374- 7/2014, version 09_2-14, dated 30-Sep-2014

Eudra-CT no.: 2014-004319-35

Version of the Report: 1.0

Date: 18-Nov-2020

I have read this report and confirm that, to the best of my knowledge it accurately describes the conduct and results of this study.

Principal Coordinating Investigator/Responsible for the study:

Prof. Dr. hab. Piotr Trzonkowski
Department of Clinical Immunology and Transplantology, Department of Immunology

Medical University of Gdańsk

_____ _____

Date Signature

Sponsor Responsible:

Prof. Dr. hab. Piotr Trzonkowski
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Medical University of Gdańsk

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Date Signature

Table of Contents

List of Tables

List of Figures

List of Abbreviations and Definitions of Terms

The following abbreviations are used in this study report:

Abbreviation or Term	Definition/Explanation
ADR	Adverse Drug Reaction
AE	Adverse Event
AESI	Adverse Events of Special Interest
ALQ	Above the upper Limit of Quantification
ANCOVA	Analysis of Covariance
AUC	Area Under the Curve
b.w.	body weight
BMI	Body Mass Index
BLQ	Below the lower Limit of Quantification
CI	Confidence Interval
CRF	Case Report Form
CRP	C-reactive Protein
CRO	Clinical Research Organization
CTP	Clinical Trial Protocol
CV	Coefficient of Variation
DCs	Dendritic cells
DDI	Daily Dose of Insulin
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus

eCRF	electronic Case Report Form
e.g.	for example (lat.: exempli gratia)
EOS	End of Study
EU	European Union
FDA	Food and Drug Administration
GAD	Glutamate decarboxylase
GCP	Good Clinical Practice
HbA1c	Glycosylated hemoglobin A1c
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
i.e.	that is (lat.: id est)
IAA	Islet Antigen-2 Antibody
IB	Investigator Brochure
ICA	Islet Cell Antibody
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IDF	International Diabetes Federation
IEC	Independent Ethics Committee
IgA, IgM	Immunoglobulin A, Immunoglobulin M
IMP	Investigational Medicinal Product
IPEX syndrome	Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome

ITT	Intention-to-treat
IV	Intravenous
kg	kilogram
LB	Laboratory domain
LLQ	Lower Limit of Quantification
LSmeans	Least Squares means
m	month
mAb	monoclonal Antibody
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
Min	Minimum
min	minute
MMTT	Mixed Meal Tolerance Test
MP	Macrophages
N	Number of patients
n	number of observations
NOD mice	Non-Obese Diabetic mice
PI	Principal Investigator
PT	Preferred Term
PP	Per-Protocol

QoL	Quality of Life
REML	Restricted Maximum Likelihood Estimation
RMANCOVA	Repeated Measures Analysis of Covariance
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SGLT2	Sodium-glucose co-transporter 2
SI	Système international d'unités
SOC	System Organ Class
SPC	Summary of Product Characteristics
T1D	Type 1 diabetes
T1DM	Type 1 Diabetes Mellitus
TB	Tuberculosis
TEAE	Treatment-Emergent Adverse Event
Teff	Effector T cells
Th1/Th2/Th17	T helper cells 1/T helper cells 2/T helper cells 17
TMF	Trial Master File
Tregs	T regulatory cells

ULQ	Upper Limit of Quantification
UCK	University Hospital Centre in Gdańsk
WHO	World Health Organization
WHO ATC 2020	World Health Organization Anatomical Therapeutic Chemical classification system 2020

Ethics

Independent Ethics Committee and/or Institutional Review Board

The protocol, protocol amendments, Informed Consent Form (ICF), Investigator Brochure (IB), and other relevant documents were reviewed and approved by the Independent Bioethics Committee for Scientific Research at the Medical University of Gdańsk (approval number NKBBN / 374 / 2012 with correction NKBBN / 374-7 / 2014 dated 21.01.2014) before the study was initiated.

Any amendments to the protocol required approval by the same Committee before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study patients.

Ethical Conduct of the Study

This study was conducted in accordance with the protocol and consensus ethical principles derived from international guidelines including the Declaration of Helsinki, the applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines and the respective national legal requirements.

The Principal Investigator (PI) agreed, when signing the Study Protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of GCP and conduct the study in accordance with the current revision of the Declaration of Helsinki.

Participant Information and Consent

An ICF explaining the procedures of the study, including the potential hazards, was reviewed and approved by the IEC before its use.

The investigator or his/her representative explained the nature of the study to the patient and his/her parents or legally authorized representative and answered all questions regarding the study.

Patients were informed that their participation was voluntary. Each patient and their legally authorized representative had ample opportunity to ask questions and was assured of the right to withdraw from the study at any time without any disadvantage and without having to provide a reason for this decision. Patients and their parents or legally authorized representative were required by the researcher to sign a statement of informed consent before being enrolled in the study. Consent was discussed during the initial clinical visit and before each study procedure commenced. Assurance that the patient and their parents understood and gave consent to the study was obtained by routine evaluation of the patient’s mental capacity as part of their routine physical examination. The authorized person obtaining the informed consent was also instructed to sign the ICF. A copy of the ICF(s) was provided to the patient and their parents or legally authorized representative.

After obtaining consent, the researcher informed the coordinator about the inclusion of the patient in the study. In case of withdrawal of consent by the patient or their legally authorized representative, the researcher informed the coordinator and made the appropriate entry in the

Case Report Form (CRF). All the patient's data were then removed by the coordinator from the study database.

A sample ICF and written information given to the patients are located in the trial master file (TMF). The original copy of the informed consent was kept in a confidential file in the Investigators center records. An example of the patient information sheet and consent form can be found in Appendix 16.1.3.

1. INTRODUCTION

1.1. Diabetes mellitus Type 1 (T1DM) - epidemiology

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder characterized by the progressive loss of pancreatic β-cell function, (1) eventually culminating in patients' dependence upon exogenous insulin to control blood glucose. The disease usually begins before the age of 35 and in most cases has an onset before 18 years. Short-term (hypoglycemia) and long-term (nephropathy, retinopathy, neuropathy, and others) complications often arise in T1DM patients as result of the inadequate control of glucose homeostasis. Moreover, insulin-induced severe hypoglycemia is often associated with brain damage (2), coma, and even death (3).

The International Diabetes Federation (IDF) estimated the global prevalence of diabetes in adults (aged 20-79) increased from 151 million in 2000 to 463 million in 2019 (4) with approximately 10% of these patients being affected by T1DM. According to the same report, the number of children and adolescents (0-19 years old) suffering from T1DM1 has reached more than 1 million and, if the current trends continue, the number of these cases is expected to increase by more than 100 000 every year. In Europe, the prevalence of T1DM in children is doubling every 25 years and currently causes an average loss of 11–12 years in life expectancy (5). In the United States, T1DM is diagnosed in 15/100 000 children, and pooled data from five centers for children and adolescents under 20 years of age indicated an annual increase of 1.8% (95% confidence interval [CI] from 1.0% to 2.6%) from 2002–2012 after adjustment for age, sex and race or ethnic group (6).

In Poland, the incidence rate in T1DM shows regional differences and has been shown to fluctuate in recent years, ranging from 5 / 100,000 and 6.18 / 100,000 in the Lublin macro region and the ex-Rzeszów province, respectively, to 9.2 / 100,000 in the Pomeranian province and Lodz region, showing constant, systematic growth (even by 100% in 10 years for the Lodz region) (7). The mean incidence of T1DM among polish children aged less than 15 years for the period 1989–2012, when standardized for age and gender, was 12.72 per 100 000 persons/year (95% CI from 11.35 to 14.21 per 100 000 persons/year). During these 24 years, the overall incidence rate in Poland increased more than four-fold, from 5.36 to 22.74 per 100 000 persons/year (8).

1.2. Management of T1DM

To date, no approved cure exists for T1DM. The treatment primarily consists of administration of exogenous insulin via frequent daily injection or continuous subcutaneous infusion in combination with lifestyle management. Recombinant human insulins with kinetics closer to normal insulin secretion have been developed over the years, and combinations of insulins that more closely mimic normal insulin secretion are now widely used (9, 10). However, the management of insulin replacement therapy is often challenging and complicated for patients and their families, even in spite of the encouraging progresses made in the development of tools aimed at helping to achieve better glycemic control (e.g., insulin management applications, wearable continuous blood glucose meters, automated text messages, etc.) (11). Moreover, insulin replacement therapies hardly prevent the major long-term complications that significantly deteriorate patients' quality of life and shorten their life expectancy.

More proactive treatment approaches aimed at preventing disease onset and preserving or restoring β-cell destruction have been explored. The current development of alternatives to exogenous insulin replacement includes immune modulation with monoclonal antibodies (i.e. anti-CD3 (12), anti-IL-1 (13), anti-CD20 (14)), the inhibition of sodium-glucose co-transporter 2 (SGLT2) (15), stem cell mobilization (16), islet transplantation (17), β-cell encapsulation (18), and adoptive transfer with regulatory T cells (Tregs) (19, 20). However, to date, none of these attempts has been successful in routine use due either to a lack of efficacy or unacceptable side effects, and strategies to prevent or delay T1DM in youth therefore remain elusive. Hence, new alternative methods of primary prophylaxis with an improved expected benefit/risk balance represent an unmet medical need.

1.3. Rationale of the study design

1.3.1. Regulatory T cells

Tregs represent a special population of immune system cells, that negatively modulate the function of other immune cells, including dendritic (DCs), macrophages (MP) and effector T cells (Teff), to protect the body against auto aggression. The role of Tregs in diabetes has been demonstrated in both animal models and humans. A lack of these cells or their inadequate function causes the development of autoimmune diseases, including T1DM, as in the case of immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, which is caused by a mutation in Foxp3, a master regulator gene required for the development and function of Tregs (21). In a preclinical model, it was shown that the administration of Tregs to non-obese diabetic (NOD) mice naturally predisposed to diabetes development could prevent the onset of the disease (22) and inhibit the progression of pathological lesions in the pancreas after disease onset (23). Since the protective role of Tregs in T1DM has been suggested, several clinical studies have attempted to potentiate their activity and/or expand them *in vivo* with the aim of preserving β-cells in recent-onset T1DM (24-26).

In the sponsor's clinical experience, the administration of Tregs is important for delaying the development of T1DM in young patients. In 2012, the sponsor presented the first promising results in children with recently diagnosed T1DM treated with Tregs expanded *ex-vivo* (Study TregVac) (20). This phase I study showed that β-cell function was preserved after a single infusion of 10 or $20x10^6$ per kg b.w. expanded autologous Tregs. Moreover, increasing the total dose to up to $30x10^6$ per kg b.w. of expanded Treg cells *via* double infusion improved metabolic outcomes at one-year follow-up while maintaining overall safety (27). Strikingly, the metabolic outcomes at one year of follow-up were the best in the group that received the highest doses of Tregs. Moreover, the best responders were children with short disease duration at the time of inclusion, suggesting that early administration of repetitive doses of Tregs might improve the clinical outcome of this therapy. However, despite these promising results and the fact that no adverse effects, such as serious infections or episodes of acute hyper- or hypoglycemia, were reported after Tregs administration, the disease eventually progressed, with all the patients who became all insulin-dependent within 2 years from study inclusion (28).

1.3.2. Anti-CD20 monoclonal antibody

Rituximab is a chimeric mouse/human anti-CD20 monoclonal antibody (mAb) currently approved in the European Union (EU) for the treatment of blood cancers (non-Hodgkin's lymphoma, chronic lymphocytic leukemia) and some inflammatory conditions (severe rheumatoid arthritis, granulomatosis with polyangiitis, microscopic polyangiitis and pemphigus vulgaris) (29).

Rituximab targets B lymphocytes, which are widely considered to play a role in the pathogenic processes leading to T1DM. Through epitope spreading, B lymphocytes might in fact increase the rate and range of islet autoimmunity and consequently increase the risk of development of T1DM (30). The deletion of B lymphocytes has been demonstrated to arrest disease development at a pre-insulitis stage in NOD mice (31). In addition, in the same animal model, anti-CD20 mAb-mediated depletion of B cells was effective in reversing hyperglycemia at onset (32, 33). The first phase 2 clinical trial investigating the therapeutic effect of rituximab in patients with recent-onset T1DM was published in 2009 and showed that, among patients treated with rituximab, β-cell function was preserved at 1 year after treatment (14). However, like several other immunotherapeutic approaches tested in recent-onset T1DM patients, rituximab was demonstrated to delay the fall in C-peptide but did not appear to fundamentally alter the underlying pathophysiology of the disease (34).

1.3.3. Combined Treg/Rituximab therapy

The present study TregVac 2.0 was designed to combine the two therapeutic approaches described above, i.e. adoptive transfer of *ex-vivo* expanded Tregs and anti-CD20 antibody rituximab administration, to simultaneously target two of the central pathways in the pathogenesis of diabetes.

Patients were recruited at an early phase of the disease and treated by repetitive administration of Tregs and anti-CD20 antibody rituximab with the aim of significantly prolonging insulin independence and further delaying disease onset.

This report presents the study results as of the final release date of the clinical database (cut-off date 30-Nov-2019).

Any study-specific information potentially originating after the registration of this report will be documented outside of this report.

2. STUDY OBJECTIVES AND ENDPOINTS

Table 2-1: Objectives and endpoints

Objectives	Endpoints
Primary	
• The primary objective of the study is to assess the safety and efficacy of the treatment in individual patient groups treated with Tregs or the combination of Tregs and anti-CD20 antibody rituximab	• C-peptide level (fasted/post-mixed meal tolerance test [MMTT] stimulation and after glucagon test) 2 years (week 104) after first dose of Tregs: clinical endpoint • Exogenous daily insulin dose per kg of body weight (DDI) 2 years (week 104) after the first dose of Tregs: clinical endpoint • Number of treated patients in remission 1 (week 52) and 2 (week 104) years after first dose of Tregs (remission defined as the number of patients with exogenous daily insulin dose lower than 0.5 U/kg/day and HbA1c lower than 6.5%): clinical endpoint • Number of adverse events reported 2 years (week 104) after the first dose of Tregs: safety endpoint
Secondary	
• To assess hypersensitivity reactions and immunosuppressive side effects Tregs or Tregs in combination with anti-CD20 rituximab antibody	• Assessment of the occurrence and severity of side effects directly related to Tregs (hypersensitivity reactions, injection-site thromboembolic events) (Tregs administration only) and blood sampling (>2g/dL drop in hemoglobin levels) on the day of Tregs administration (Day 0, Day 90) • Assessment of the occurrence and severity of effects directly related to anti-CD20 rituximab antibody administration (hypersensitivity reactions) (anti-CD20 antibody administration only) on days with anti-CD20 antibody administration (Days 14, 22, 29, and 36) • Assessment of the occurrence and severity of side effects associated with administration of Tregs or anti-CD20 rituximab antibody, primarily immunosuppressive effects: occurrence of infections of any etiology and *de novo* tumors detected • Any serious adverse event in two or more patients with confirmed association to the administration of therapy • These four secondary safety endpoints will be documented as adverse events (AEs) of special

• To investigate further efficacy and side effect parameters for the investigational medicinal product (IMP) given	interest (AESI) and related treatment-emergent adverse events (TEAEs), where appropriate • C-peptide level (post MMTT stimulation and in glucagon test) – (week 12, 26, 52, 78, 104) • Exogenous insulin dose per kg of body weight – (week 2, 3, 4, 5, 12, 14, 26, 39, 52, 65, 78, 92, 104) • The proportion of insulin-independent patients – (week 52, 104) • The proportion of patients with DDI ≤ 0.5UI/kg b.w. – (week 52, 104) • HbA1c level (%) – (week 2, 5, 12, 26, 39, 52, 65, 78, 92, 104) as glycemic control (fasting average of 7 days) • The amount and intensity of side effects of therapy – (week 52 and 104) • Quality of life (QoL) (week 5, 12, 14, 26, 52, 104) based on the QoL Questionnaire (Annex 4 of the Study Protocol, Appendix 16.1.1) • Peripheral blood lymphocyte immunophenotype: (week 2, 5, 12, 14, 26, 39, 52, 65, 78, 92, 104) with basic phenotype results determined as part of this analysis and additional results that will be analyzed and documented in a separate report

3. INVESTIGATIONAL PLAN

The protocol and all its amendments can be found in Section 16.1.1. A copy of a sample blank CRF can be found in Section 16.1.2.

3.1. Overview of Study Design

This was a phase 1/2, prospective, randomized [1:1], open label (regarding Tregs treatment, control group) and single-blinded (patient blinded, regarding anti-CD20 antibody rituximab treatment groups), multi-center clinical study performed in children and adolescents with type 1 diabetes with two parallel groups and a treatment-free control arm group (total N=45, Tregs group N=15, Tregs + anti-CD20 rituximab group N=15, treatment-free control group N=15).

The total duration of the study was up to 42 months divided as follows:

Inclusion of patients: 18 months

Observation time (for each patient): 24 months

Patients aged 8-16 years that were diagnosed with T1DM no more than 2 months before the application of the first dose were included in the study.

Patients who fulfilled all of the inclusion criteria and none of the exclusion criteria and who provided their informed consent received a sequential number (1 to 45) and were randomly [1:1] allocated to one of the treatment groups (Tregs or Tregs plus anti-CD20 rituximab antibody treatment).

Patients who, despite meeting the inclusion criteria, for various reasons did not receive any treatment (either Tregs or anti-CD20 rituximab antibody treatment) but had agreed to routine assessment for the purpose of the study were offered to continue to participate in all of the visits as part of the control group of the trial.

Patients who started treatment but were disqualified for various reasons were included in the observation group.

Consequently, the study groups were defined as shown in Table 3-1.

Table 3-1: Treatment Groups

Treatment group (by order of appearance in the clinical trial outputs)	Treatment
Tregs+anti-CD20 antibody rituximab	Tregs infusion at time "0" + 4 doses of anti-CD20 antibody rituximab; second infusion of Tregs at time "90 ± 30 days"
Tregs	Tregs infusion at time "0" + 4 placebo doses of anti-CD20 antibody; second infusion of Tregs at time "90 ± 30 days"
Control	No intervention (no Tregs infusion, refusal of blood donation or treatment with the proposed scheme; or excluded from blood drawing due to inappropriate venous access) but agreed to routine testing
Observational group	Patients exposed to study treatment but censored due to failure to meet the criteria for individual groups

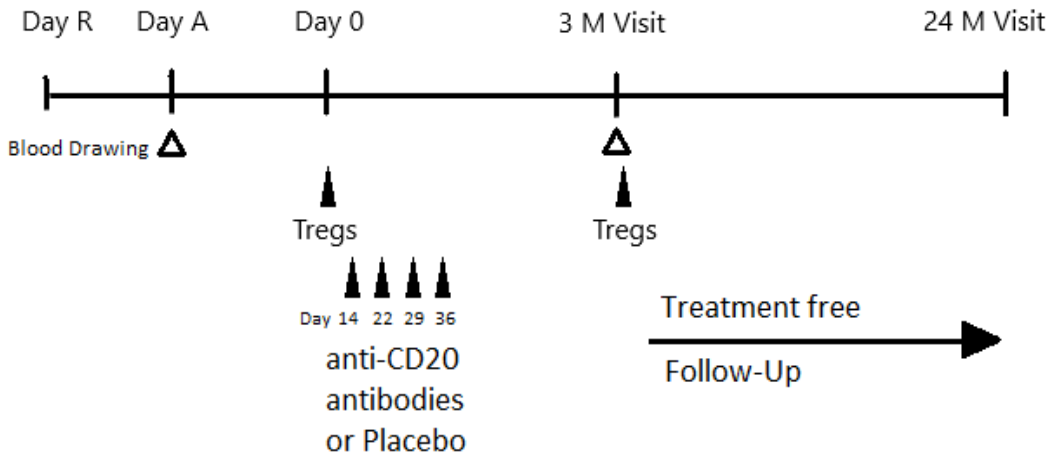
Abbreviations: Tregs: T regulatory cells

Altogether, 45 patients were intended to be enrolled in the trial, with 15 patients in each of the groups (Tregs+CD20, Tregs, Control). Finally, the study enrolled the following patients into the ITT (intention-to-treat) set (completed the trial without any major protocol violation and had the month 24 assessment of the primary efficacy variable): 12 patients in Tregs+CD20 group, 13 patients in Tregs group, 11 patients in Control group.

The study time plan is summarized in Figure 3-1. Study intervention consisted of two infusions of expanded Tregs (on Day 0 and Day 90) and four administrations of anti-CD20 antibody or placebo (on Days 14, 22, 29, and 36) followed by a 21-month follow-up period.

Study start was defined as the time when the first patient signed the ICF, and study end was defined as the time when the last patient had the last contact.

Figure 3-1: Study Time Plan



Abbreviations: Tregs: T regulatory cells

3.1.1. Discussion of Study Design

The scientific rationale for features of the study design, including the chosen control groups, doses, and endpoints, as applicable, are discussed in the Study Protocol (Appendix 16.1.1).

3.1.2. Changes in Study Conduct

There were no changes in the conduct of the study.

3.2. Investigators and Study Administrative Structure

Medical University of Gdańsk - Skłodowskiej-Curie 3a, 80-210 Gdańsk, Poland - is the sponsor of this study.

Table 3-2 lists the study administrative activities and the names of vendors, laboratories, and Clinical Research Organizations (CROs) used by the sponsor during the conduct of the study.

This was a multicenter study conducted at 4 study centers in Poland. Only investigators qualified by training and experience were selected as appropriate experts to investigate the study treatment. At each center, the PI was responsible for the study. All laboratory determinations were performed by a central laboratory at the Department of Pediatrics, Diabetology and Endocrinology of the University Clinical Center, Gdańsk.

Prof. Malgorzata Myśliwiec was appointed as a principal investigator. Prof. Piotr Trzokowski was appointed as a coordinating investigator for the review and approval of this study report. The signature of the coordinating investigator is located in Appendix 16.1.5.

Lists of each center's PI, each sponsor's personnel, and other important participants in the study are located in Appendix 16.1.4.

Table 3-2: Study Administrative Structure

Function	Name of Responsible Company/Organization
Statistical analysis	ICRC-Weyer GmbH, Bölschestraße 35, 12587 Berlin, Germany
Preparation of CSR	ICRC-Weyer GmbH, Bölschestraße 35, 12587 Berlin, Germany
IMP	
IMP production	IDEC Pharmaceuticals, San Diego, CA
IMP management and shipment:	Genentech, Inc., South San Francisco, CA

Abbreviations: CSR: clinical study report, ICRC: Independent Clinical Research Consulting, GmbH: *Gesellschaft mit beschränkter Haftung*, IMP: investigational medicinal product

3.3. Selection of Study Population

3.3.1. Inclusion/Exclusion Criteria

Enrolled in this study were patients with type 1 diabetes. Detailed inclusion and exclusion criteria are provided in section 5.1 of the Study Protocol (Appendix 16.1.1).

3.3.2. Removal of Participants from Intervention or Study

The specific criteria and procedures for early discontinuation from study intervention(s) or withdrawal from the study are described in the Statistical Analysis Plan (Appendix 16.1.9).

3.4. Study Intervention

3.4.1. Study Interventions Administered

The study interventions administered are outlined in Table 3-3. The methods used for the preparation, storage, and administration of the study interventions are provided in section 5.3 of the Study Protocol (Appendix 16.1.1).

The justification for the doses selected is described in the justification for dose section of the protocol (Appendix 16.1.1).

The phase I/II TregVac study showed that β-cell function was preserved after a single infusion of 10 or $20x10^6$ per kg b.w. expanded autologous Tregs. Increasing the total dose to up to $30x10^6$ per kg b.w *via* double infusion improved metabolic outcomes at one year of

follow-up while maintaining overall safety. Based on these results and the fact that repetitive doses of Tregs seemed to have a positive impact on clinical outcomes, in this study, patients received doses of Tregs between 10-30x10^6/kg b.w. (not more than 60x10^6/kg b.w.) in two administrations on Day 0 and Day 90.

A phase II study of the Type 1 Diabetes TrialNet showed that β-cell function was preserved over a period of one year and that a favorable safety and tolerability profile was observed in patients affected by type 1 diabetes who were treated with four administrations of anti-CD20 antibody rituximab at a dose of 375 mg/m^2 (35). Based on these findings, the same dose regimen was used in the present study.

Table 3-3: Study Interventions Administered

Dose Group	Treatment	Route of Administration	Frequency of administration	Number of subjects planned to be treated
1	2 doses of up to 30x10^6 Treg cells per kg b.w.	IV infusion	D0; D90	15
	4 doses of 375mg/m^2 of Rituximab	IV infusion	D14; D22; D29; D36	
2	2 doses of up to 30x10^6 Treg cells per kg b.w.	IV infusion	D0; D90	15
	4 doses of placebo	IV infusion	D14; D22; D29; D36	

Abbreviations: Treg: T Regulatory cell, b.w.: body weight: IV: intravenous, D: day

The manufacturing lot numbers for the study intervention rituximab dispensed in this study are provided in Appendix 16.1.6.

3.4.2. Measures to Minimize Bias

Allocation and Blinding

Patients who fulfilled all of the inclusion criteria and none of the exclusion criteria and who provided their informed consent were offered the choice of receiving the intervention Tregs or not (control group) (open label) with the information that additional treatment (anti-CD20 antibody rituximab or placebo) would be administered in the Tregs group in a randomized, patient-blind manner. Among patients assigned to the intervention group, the assignment to the anti-CD20 antibody rituximab (Tregs plus anti-CD20 antibody rituximab) group or the placebo (Tregs) group was based on a randomization procedure performed by the medical team.

Due to the methods used to produce the Tregs treatment, the randomization was performed by the team involved in the expansion of Tregs. For the same reason, the person responsible for randomization did not have access to the clinical and laboratory results collected by the team responsible for the clinical part of the study until the end of the study. The randomization followed a 1:1 scheme (Tregs) : (Tregs plus anti-CD20 antibody rituximab) and was performed as plain assignment to both treatment groups using an element of chance (coin) as documented in the CRF.

In order to ensure balance between these two treatment groups relative to the control group (Control) with no treatment, a comparable number of patients were included in the control

group. The control group (Control) included 15 patients who refused to receive any treatment but agreed to attend the follow up visits as planned in the protocol.

Patients who for various reasons failed to receive a full treatment were proposed to continue to participate in the follow-up program and to undergo the same visit plan as that of the patients who received a full treatment.

3.4.3. Study Intervention Compliance

All administrations of the study medication were done under supervision of a member of the investigator's team. This person ascertained and documented that the patient received the treatment as planned.

3.4.4. Prior, Concomitant, and Post-intervention Therapy

The medications, treatments and vaccinations allowed or disallowed before, during, and/or after study intervention, including any exceptions to these requirements, are described in Section 5.3.3 of the study protocol (Appendix 16.1.1). In order to reduce adverse effects associated with anti-CD20 antibody rituximab administration, patients were allowed an antipyretic / analgesic (e.g., 0.5 g of paracetamol 30 min before infusion) and an antihistamine (e.g., 50 mg of diphenhydramine intravenously immediately prior to infusion, 2 mg of clemastine intravenously immediately prior to infusion). Due to the possibility of hyperglycemia, premedication with glucocorticosterone was not used.

3.5. Study Assessments and Procedures

3.5.1. Planned Measurements and Timing of Assessments

The specific efficacy and safety assessments and their schedule and measurement/collection methods are described in the Procedures sections of the protocol (Appendix 16.1.1 Study Protocol). The collection and assessment of safety information during the study (evaluation, definitions, recording, and reporting of AEs and SAEs and other reportable safety events) is detailed in the AE reporting section of the protocol (Appendix 16.1.1 Study Protocol).

The schedule of assessment is presented in Table 3-4.

Table 3-4: Schedule of assessment

	R[1]	A[2] week -2	0d[3] week 0	+14d[4] week 2	+22d week 3	+29d week 4	+36d week 5	+3m[2] week 12	+3m[prim,3] week 14	+6m week 26	+9m week 39	+12m week 52	+15m week 65	+18m week 78	+21m week 92	+24m week 104
Clinical examination	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x
Exogenous insulin requirement	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x
HbA1c (%)	x	x		x			x	x		x	x	x	x	x	x	x
C-peptide	x	x		x			x	x		x	x	x	x	x	x	x
C-peptide MMTT and glucagon test	x	x[5]						x[5]		x		x		x		x
Blood test+CRP+urine test		x	x	x	x	x	x	x	X							
Quality of life	x						x	x	X	x		x				x
Immunophenotype: Tregs + B Lymphocytes		x	x	x			x	x	X	x	x	x	x	x	x	x
Autoantibodies	x	x		x				x		x		x		x		x
Cytokines		x		x			x	x		x	x	x	x	x	x	x
Molecular tests		x		x			x	x		x	x	x	x	x	x	x

Abbreviations: CRP: C-reactive protein, Tregs: T regulatory cells, HbA1c: glycosylated hemoglobin A1c, d: day, MMTT: mixed meal tolerance test, m: month
Footnotes: 1 recruitment, 2 - blood drawing, 3 - Tregs infusion, 4 – anti-CD20 antibody in TregsCD20 group +14, +22, +29, +36 day, 5 – functional tests during blood drawing

3.5.2. Appropriateness of Measures

The endpoints and measures used in this study were standard, considered to be reliable, and relevant to the objectives set forth in the Study Protocol (Appendix 16.1.1).

3.6. Data Quality Assurance

3.6.1. Study Monitoring

Study centers were responsible for recruitment and the treatment and follow up were performed in the University Hospital Centre in Gdańsk (UCK). The centers were monitored by the sponsor. One of the investigators was specifically dedicated to this function and separated from other activities during the study duration (Dr. Natalia Marek-Trzonkowska). Centers were visited at regular intervals. The monitoring was documented by signing CRFs of particular patients. In addition, eCRFs were regularly viewed by the sponsor representative. Monitors were responsible for reviewing adherence to the protocol; compliance with GCP; and the completeness, accuracy, and consistency of the data. Direct access to patient medical and laboratory records was permitted to verify entries on the CRFs. In addition, the External Committee was monitoring the study annually.

3.6.2. Investigator Meetings and Staff Training

Investigator staff training was provided by the sponsor during investigator meetings and routine monitoring visits. The sponsor organized investigator and clinical research associate meetings before study start and during the study to provide information on the investigational product, the study rationale and design, responsibilities under ICH/FDA/GCP, and training on the detailed study requirements.

3.6.3. Laboratory Procedures

A central laboratory, the Department of Pediatrics of Diabetology and Endocrinology of UCK, Gdańsk, was used to analyze blood and urine samples. Where local laboratories were used, their participation in internal and external quality control, quality assurance, and accreditation schemes was evaluated by the study monitors.

3.6.4. Investigator Responsibilities

The investigators were responsible for all data entered in the CRFs and documented their review and approval of the data, verifying the validity and completeness of the data. The investigator was responsible for appropriate retention of essential study documents.

3.6.5. Clinical Data Management

Case report form data were captured via data entry by study center personnel in a sponsor database system. Data quality checks were applied using manual verification methods. An audit trail to support data query resolution and any modification to the data was maintained.

3.6.6. Clinical Quality Assurance Audits

Quality audit assessments were not performed for this study.

3.7. Statistical Analysis

3.7.1. Statistical Analysis Plan

The planned analyses, comparisons, and statistical tests and determination of sample size are described in the final version of the SAP (Appendix 16.1.9).

All variables and analyses reported in this section are based on the final version of the statistical analysis plan (SAP v.2.0), dated 05-Aug-2020 (Appendix 16.1.9).

3.7.1.1. General statistical considerations

The statistical evaluation was performed using the Statistical Analysis System (SAS® release 9.3 or later - SAS Institute Inc., Cary, NC, USA).

All data were listed by treatment group, patient and all available time points of assessments (see Section 3.5.1 for Schedule of Assessments). The data listings included the data of all randomized patients as far as available. The observational group were flagged in the listings and included in the ITT analysis set but excluded from the PP set (Section 3.7.1.2).

Data summaries were presented by treatment group using descriptive statistics or frequency tables as appropriate and include the total number of patients (N) for each treatment group.

Categorical data were summarized in frequency tables by the number and percentage of patients in each category.

Continuous variables were summarized by descriptive statistics, including the number of observations [n], mean, standard deviation (21), minimum [Min], maximum [Max], median. Concentration data of efficacy variables were additionally summarized by geometric mean and geometric SD.

Baseline values were defined as the last scheduled assessment prior to the first administration of investigational product (Day 0). For post-dose assessments, the first scheduled value was used for summary analysis if repeated measurements were made at a particular time-point.

The planned tests of geometric mean ratios in an ANCOVA with pairwise comparisons following a one-side testing approach with an alpha level of 5% were performed as follows:

For parameters where higher result values were better:

- Tregs + anti-CD20 rituximab / Control (1-sided test for superiority using the lower 90% CI limit, threshold ratio was 1)
- Tregs / Control (1-sided test for superiority using the lower 90% CI limit, threshold ratio was 1)
- Tregs / Tregs + anti-CD20 rituximab (1-sided test for non-inferiority using the lower 90% CI limit, alpha level 5%, non-inferiority margin is 20%, i.e. critical threshold ratio is 0.8)

For parameters where lower result values were better between the following groups:

- Tregs + anti-CD20 rituximab / Control (1-sided test for superiority using the upper 90% CI limit, threshold ratio was 1)

- Tregs / Control (1-sided test for superiority using the upper 90% CI limit, threshold ratio was 1)
- Tregs / Tregs + anti-CD20 rituximab (1-sided test for non-inferiority using the upper 90% CI limit, alpha level 5%, non-inferiority margin was 20%, i.e., critical threshold ratio was 1.2)

As the tests were performed in a logical order as described above, and the overall alpha level could be controlled.

Unless otherwise indicated, all statistical analyses were pre-specified in the Clinical Study Protocol (Appendix 16.1.1) and SAP (Appendix 16.1.9). No further formal testing of statistical hypotheses were undertaken unless stated in the relevant sections of the SAP. Unless otherwise specified, there were no substitution of missing data (i.e., missing data were replaced and handled as 'missing' in the statistical evaluation).

3.7.1.2. Analysis sets

Data analysis was based on three different trial populations. The final decision on inclusion of patients in the different analysis sets was made at a data review meeting before the hard lock of the database.

Safety Set: The safety set includes all trial patients who were randomized and who received any IMP or other trial treatment. Patients in this population will be used for safety analyses and will be analyzed as treated. **Intention-to-treat (ITT) Set:** The intention-to-treat (ITT) population includes all patients of the safety set who received at least one dose of trial medication and had at least one assessment of the primary efficacy variable beyond the baseline assessment up to month 24. Patients in this population were used for the primary analysis of efficacy and were analyzed as randomized. **Per-Protocol (PP) set:** The per-protocol (PP) population includes all patients from the ITT set who completed the trial without any major protocol violation and who had the month 24 assessment of the primary efficacy variable. Patients in this population were used for a supportive analysis of efficacy and were analyzed as randomized and treated. Demographic data, baseline characteristics as well as all other variables which are not defined as efficacy or safety variables were analyzed using the safety set, ITT set and PP set. Separate summaries for each population was produced.

3.7.1.3. Handling of Laboratory Values

Laboratory parameters provided were harmonized to SI-units before data analysis, if necessary. .

The following rules were used to handle efficacy parameters (and later used for the calculation of AUC values) below the limit of quantification (BLQ) and above the limit of quantification (ALQ):

1. BLQ values were set to the lower limit of quantification (LLQ) and included in the summaries if less than 50% of all observations were BLQ.
2. BLQ values was set at "0" and included in the summaries if more than 50% of all observations are BLQ.

3. ALQ values were set to the upper limit of quantification (ULQ) and included in the summaries if less than 50% of all observations are ALQ.
4. If more than 50% of all observations are ALQ, then the parameter was listed only and not included in summary statistics.

Other laboratory values above a specific limit (listed as '>xx.xx') or below a specific limit (listed as '<yy.yy') were displayed unchanged in parameter listings but replaced by the corresponding upper (xx.xx) or lower (yy.yy) limit of the value for descriptive statistics.

3.7.1.4. Handling of missing values and outliers

There was no imputation of missing data (i.e., patients who prematurely discontinued from the study were not included in summary statistics or analyses beyond the time of discontinuation). If a patient was withdrawn from study treatment but continued to participate in the study, the data collected were reported against the treatment received by the patient. All available data were included in the analyses and were summarized to the last available visit date. Missing data were handled as missing in all statistical summaries and analyses.

Outliers were included in the derivation of the summary statistics and were not removed from the data. The inclusion of the median, Min and Max values allowed the evaluation of the impact of outliers.

Adverse events (AEs) with an unknown start date/time were assumed to be treatment-emergent if the known part of the date/time was on or after the first use of the IMP unless the end date/time was known to be before the first use of IMP. AEs commencing on the day of treatment with missing start time were assumed to be treatment-emergent. There were to be no missing values for AE severity or relationship. It was the sponsor's responsibility to ensure all AEs had assessments of severity and relationships that were completed by the investigator(s).

For previous and concomitant medication, if the medication's start date was incomplete and it was not clear whether the medication was concomitant, it was assumed to be concomitant.

3.7.1.5. Coding of Adverse Events, Medical History and Concomitant Diseases

AEs as well as previous and concomitant diseases were collected according to 'Common Terminology Criteria for Adverse Effects CTCAE' ver. 3.0 and then coded according to the Medical Dictionary for Regulatory Activities (MedDRA, version 21.0 or higher). Previous and concomitant medications were coded according to the World Health Organization Anatomical Therapeutic Chemical classification system (WHO ATC 2020), if applicable. All listings and tables displaying terms of coding dictionaries include a footnote presenting the version of the dictionary used.

3.7.1.6. Sample size and power

For the present study, a sample size of 13 patients per randomized treatment arm was deemed sufficient to detect a 20% difference in the geometric mean ratio of AUC (0-240 min) of C-peptide, with a pairwise comparison following a non-inferiority one-side testing approach and

an alpha value of 5% with a power estimate of > 80% assuming a CV of 0.2 if the real geometric mean ratio was 1 (see Figure 3-2).

Assuming a drop-out rate of approximately 10%, this means this sample size could be achieved by enrolling 15 patients per treatment group.

Similar assumptions applied for the secondary endpoints in an exploratory manner.

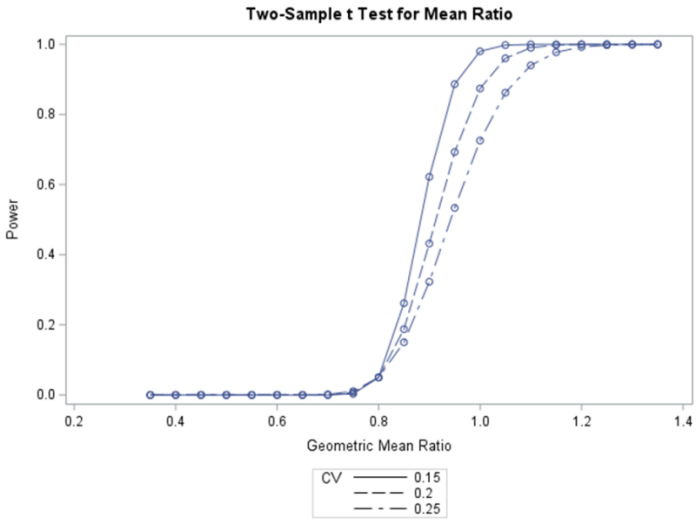


Figure 3-2. Power of detecting a 20% difference in geometric mean ratio (alpha = 5%) with a sample size of 13 subjects

3.7.1.6.1. Exploration of sample size and power

Using the same assumptions provided in Section 6.1 of the SAP, an exploration of the impact of not achieving the proposed number of patients per randomized treatment arm in this one-side testing approach is presented in Figure 3-3.

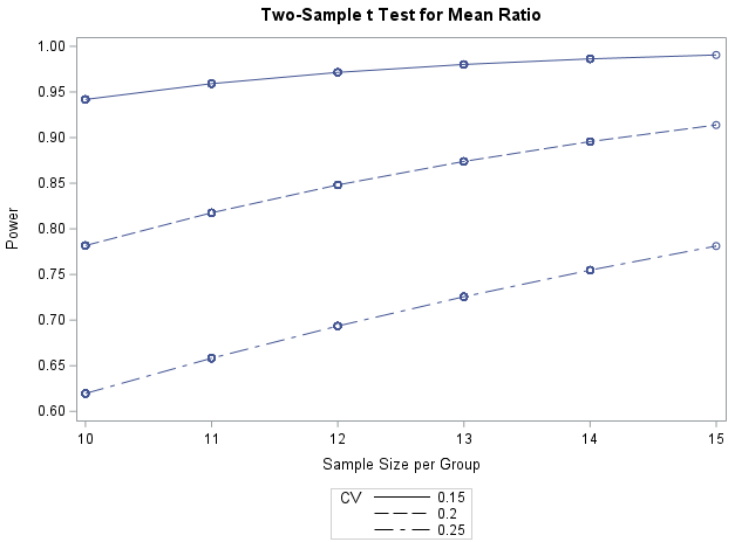


Figure 3-3. Resulting power of detecting a 20% difference in geometric mean ratio (alpha = 5%)

As presented, the impact of a reduced sample size on the power of detection for a 20% difference in the geometric mean ratio between the treatment groups was limited, and a power

level of around 80% could be achieved if the CV was <= 20% and the sample size bigger than 11 subjects per treatment group.

3.7.1.7. Demographics and baseline characteristics

3.7.1.7.1. Participant disposition

Patient disposition was listed based on individual study examination dates, where available. Disposition of all randomized patients was also summarized, providing the following information by treatment group:

- number of randomized patients
- number and percentage of patients who discontinued early with reason for early discontinuation

The number and percentage of patients randomized, patient analysis set (Safety, ITT and PP Set), and treatment status (completed, discontinued) was summarized by treatment group and overall. The assignment of patients to the defined analysis populations (see Section 3.7.1.2) was presented in a frequency table. Moreover, the analysis populations were characterized according to age group and gender categories. The number and percentage of patients in each category was also presented.

By-patient listings were provided for discontinued patients and patients excluded from the ITT, PP and Safety Sets.

3.7.1.7.2. Protocol deviations

Any incident that was reported involving noncompliance with the protocol that had no significant effect on the patient's rights, safety, or welfare or the integrity of the data was considered a protocol deviation and analyzed in a data review meeting for possible impact on study results before database closure. Patients with important deviations from the protocol could be excluded from predefined analysis sets (i.e. the PP Set) depending on the specific conditions. A listing of deviations, their assessed importance, and exclusion of patients from predefined analysis sets was provided,

Any deviations from the protocol which occurred during the study were listed individually (Listing 16.2.2.1) and categorized as follows:

- inclusion/exclusion criterion violations
- study drug exposure deviations (e.g. dose, treatment)
- time deviations
- procedural deviations
- other deviations.

Where appropriate, further categories could be created to allow a clear assessment of deviations.

3.7.1.7.3. Demographic Data

A by-patient listing of demographic data (gender, age at screening, race, height, weight, body mass index (BMI)

BMI=weight (kg) / (height (m))^2

and of possible childbearing potential (criteria: female and > 15 years, as to CTP) will be provided (Listing 16.2.4.1). Demographic data will be additionally summarized with descriptive statistics (age, height, weight, BMI) or frequencies (gender, childbearing potential and race) by treatment group as appropriate (Tables 14.1.3.1 and 14.1.3.2).

3.7.1.7.4. Baseline characteristics

Medical history data, i.e. disease diagnosis date, duration since diagnosis date and duration since diagnosis, were listed by patients. These variables were also summarized with descriptive statistics and by number of months since diagnosis.

Laboratory parameters (childbearing potential, glycemia test results at onset, autoantibodies and C-peptide test results) collected at baseline were also listed by patient.

3.7.1.7.5. Previous and concomitant medication

Previous and concomitant medications were not specifically collected during the trial and only generally mentioned as specific treatment given for AE handling. These cases were listed and for concomitant medications also summarized with descriptive statistics and by number and percentage of patients by treatment.

3.7.1.8. Analysis of efficacy variables

3.7.1.8.1. Primary efficacy parameters

All analyses of primary efficacy variables were performed on the ITT analysis set.

AUC of C-peptide (MMTT, 0-240 min) at 24-m (week 104) visit

The AUC of C-peptide levels (MMTT, 0-240 min) obtained on Day 0, +3 m, +6 m, +12 m [week52], +18 m visits and at the end of the study (+24 m visit) was listed by patient and visit.

The AUC of C- peptide levels was calculated based on C-peptide level measurements at 0, 15, 30, 60, 90, 120. 150, 180, 210 and 240 min after the start of MMTT using the linear trapezoidal rule.

Descriptive statistics for the AUC values of C-peptide levels and changes from baseline (Day 0) were provided for the treatment groups. The mean AUC values of C-peptide levels (MMTT, 0-240 min) at all visits were presented graphically in the same graph overlaid for treatment group using a mean-time-plot. Geometric mean time plots of AUC values of C-peptide levels (MMTT, 0-240 min) were provided.

An analysis of covariance (ANCOVA) of logarithmized AUC of C-peptide levels (MMTT, 0-240 min) at +24 m [week 104] visit was performed using the logarithmized AUC of C-peptide levels (MMTT, 0-240 min) at baseline (Day 0) as a continuous covariate and age group (either <=12 years or >12 years), gender and treatment as fixed categorical effects. Treatment

difference estimates and their 90% confidence intervals (CI) using a one side significance threshold of α=0.05 were provided. These values from the ANCOVA were back-transferred from the logarithmic scale to obtain geometric mean ratios between the treatment groups [Tregs+ anti-CD20 antibody rituximab]/Control and Tregs/Control and their 90% CIs for a one-sided test of superiority towards the control group (1 not included). For the ratio of the group comparison Tregs/[Tregs+ anti-CD20 antibody rituximab] a one-sided non-inferiority test using a 20% threshold was provided. These tests were performed in the following logical order:

1. [Tregs+ anti-CD20 antibody rituximab] /Control (1-sided test for superiority, alpha level 5%)
2. Tregs/Control (1-sided test for superiority, alpha level 5%)
3. Tregs/[Tregs+ anti-CD20 antibody rituximab] (1-sided test for non-inferiority, alpha level 5%, non-inferiority margin 20%, i.e. critical ratio level 0.8).

The overall alpha level of 5% for these three consecutive one-sided tests was controlled. All treatment comparison geometric mean ratio result estimates and their 90% CIs were displayed in a Forest Plot.

SAS Proc Mixed with restricted maximum likelihood estimation (REML) was used. The Kenward-Roger approximation was used to estimate denominator degrees of freedom for tests of fixed effects. The assumptions of the model, including normality, were evaluated using residual and other diagnostic plots of model fit.

In case the check for normal distribution of data was not given for logarithmized values (cross-checked with QQ-plot or Shapiro-Wilk test for normality (i.e. both Shapiro-Wilk test p-values $\geq$ 5%)), the results of the nonparametric randomization-based analysis of covariance in SAS (36). were alternatively provided.

Subgroup analyses were used to test the influence of gender and age group and potentially other variables (e.g., autoantibodies, cytokines) in separate analyses with a model specific for these comparisons. If appropriate, these analyses were displayed in the table and Forest plot of the variable.

AUCs of C-Peptide (MMTT) values at the other follow up timepoints starting from month 3 were analyzed as secondary objectives in an RMANCOVA.

C-Peptide (Glucagon Test, 0 and 6min) at 24-m [week 104] Visit

An ANCOVA of logarithmized values of the +24 m [week 104] visit similar to that used for the AUC of MMTT C-peptide levels was also performed for the AUC of C-peptide levels of the Glucagon tests (for the AUC calculated between 0 min and 6 min using the linear trapezoidal rule) and also separately for concentrations at the 24-m visit.

Descriptive statistics for the AUC of C-peptide levels were provided for the treatment groups by visit and treatment. C-peptide levels (Glucagon Test, 0 and 6min) at the +24-m [week 104] and other visits were also summarized.

The AUC of C-peptide levels was presented graphically by visit and with overlaid treatment groups using mean concentration time plots +/-SD and geometric mean concentration time plots with 90% CIs. The C-peptide levels (Glucagon Test, 0 and 6 min) were also presented

graphically using mean +/-SD concentration time plots and geometric mean concentration time plots with 90% CIs.

All (ANCOVA) treatment comparisons for AUC of C-peptide levels of the glucagon test (0-6 min) were displayed with geometric mean ratio result estimates and their 90% CIs in a Forest Plot and also separately for concentration levels.

All follow up visits (starting from month 3) were analyzed as secondary objectives in an RMANCOVA model.

C-Peptide (MMTT, 0-240min) at 24-m [week 104] Visit

C-peptide levels measured at 0, 15, 30, 60, 90, 120. 150, 180, 210 and 240 min after the start of MMTT performed on Day 0, +3 m, +6 m, +12 m, and +18 m visits and at the end of the study (+24 m visit) were listed by patient, time point and visit and summarized by descriptive statistics by treatment and visit and timepoint after start. Mean concentration by visit plots were provided for each timepoint after start with treatments overlaid (arithmetic mean, ±SD; geometric mean ±90% CI.

An ANCOVA of logarithmized values of the 24-m [week 104] visit similar to that used for the AUC of MMTT C-peptide levels was also performed for the C-peptide levels of the MMTT tests separately for each timepoint.

All (ANCOVA) treatment comparisons geometric mean ratio result estimates and their 90%CIs were displayed in a Forest Plot.

All other follow up visits (starting from month 3) were analyzed as secondary objectives in an RMANCOVA model.

C-Peptide (Fasted, 24 m [week 104] Visit)

C-peptide levels (fasted, 24-m [week 104] visit) were listed by patient and visit and tabulated by visit and treatment for each analysis set.

For the C-peptide level (fasted, 24-m [week 104] visit) log transformation of fasted C-peptide values, a similar analysis approach was performed using the log baseline value of the C-peptide level (fasted, Day 0) as a covariate and age group, gender and treatment as fixed categorical effects for the treatment group comparison with an ANCOVA. Treatment difference estimates and their 90% CI using a one side significance threshold of α=0.05 were provided. Geometric mean ratios for the comparison between the treatment groups were obtained via back-transformation of analysis results and displayed in a Forest plot. If deemed appropriate, a display of subgroup analyses for gender, age group and other potential influencing factors was added to this graph.

Mean concentration by visit was plotted with treatments overlaid (arithmetic mean, +-SD; geometric means and their 90% CIs.

All other follow up visits (starting from month 3) were analyzed as secondary objectives in an RMANCOVA model.

Exogenous Daily Insulin Dose (24m [week 104] Visit)

The exogenous daily insulin dose was listed by patient and visit.

For the daily insulin dose per kg b.w. (DDI) at the 24-m and other visits, the original values were tabulated by treatment for each analysis set. An ANCOVA performed using baseline value (Day 0) as the covariate and age group, gender and treatment as fixed categorical effects was performed for the treatment group comparison on original (not log transformed) values. LSmeans and 90% CIs were provided as follows for the group differences: Tregs - control, [Tregs+ anti-CD20 antibody rituximab]-control and Tregs-[Tregs+ anti-CD20 antibody rituximab]. The ANCOVA model used baseline values of DDI at recruitment and age and gender as covariates for the treatment comparison and is displayed in a plot with Estimates and 90% CIs.

Mean concentration was plotted by visit with treatments overlaid (arithmetic mean, +-SD, mean estimates and their 90% CIs).

Remission (12-m [week 52] and 24-m [week 104] Visit)

Remission after one year and two years (defined as DDI <0.5 U/kg/day and HbA1c <6.5%) was listed by patient and visit and assessed with the exact permutation test on the 'Treatment x Remission(y/n)' - contingency table to obtain p-values for all three treatment comparisons. This was provided separately for the one-year and two-year assessments.

The survival analysis for remission starting from month 3 was also provided.

A Kaplan-Meier plot of clinical remission is displayed with treatments overlaid, by time (weeks) starting from the 3-m visit.

3.7.1.8.2. Secondary efficacy parameters

Depending upon the availability of the underlying data, the following analysis of secondary efficacy parameters may have been undertaken using the ITT set.

AUC of C-Peptide (MMTT, 0-240-min, All Visits)

The AUC of C-Peptide (MMTT, 0-240 min) was listed by patient and visit and tabulated by visit and treatment group using descriptive statistics.

For comparison of treatment C-peptide (MMTT) AUCs from month 3 to month 24, a repeated measurement mixed model RMANCOVA for the logarithmized AUC of C-peptide (0-240 min) values, with log baseline values (prior to first treatment, Day 0) used as the continuous covariate and gender, age group (either <=12 years or > 12 years), and treatment*visit (Days) interaction used as fixed categorical effects, was performed for the within-subject repeated measurements during follow up visits, i.e. each follow-up visit AUC was included in this analysis.

SAS Proc Mixed with restricted maximum likelihood estimation (REML) and an unstructured within-patient covariance structure was used. If this model failed to converge, a first order autoregressive (AR(1)) covariance structure was used. If this model failed to converge, a simpler model (e.g. without age group or gender as a fixed effect categorical variable) was tested. The Kenward-Roger approximation was used to estimate denominator degrees of freedom for tests of fixed effects. The assumptions of the model, including normality, were evaluated using residual and other diagnostic plots of model fit.

From this model, least squares means, standard errors, treatment differences in least squares means, and 90% confidence intervals were estimated for each time point. The overall test for treatment group by visit interaction (adjusted for covariates) and treatment comparisons for each visit were provided with their estimates and 90% CIs on an exploratory basis. Back transformation from the logarithmic scale was performed to display the corresponding treatment ratios and CIs for [Tregs+ anti-CD20 antibody rituximab] /Control, Tregs/Control and Tregs/ [Tregs+ anti-CD20 antibody rituximab] at each follow up visit assessment. A Forest plot was used to display back-transformed treatment comparison estimates and 90% CIs. If deemed appropriate a display of subgroup analyses for gender, age group and other potential influencing factors was added to the tables and graphs for this analysis.

C-Peptide (MMTT, All Visits)

C-Peptide (MMTT Test, month 3 to month 24) was listed by patient and visit and tabulated in a summary table by visit and treatment.

For comparison of treatment C-peptide (fasted) levels from month 6 to month 24, a mixed model repeated measurement RMANCOVA for logarithmized C-peptide(fasted) values was performed using the interaction term visit*treatment, sex and age group as fixed categorical effects, and the respective log baseline value (prior to first treatment, Day 0) as a continuous covariate for the within-subject repeated measurements during follow-up by timepoint (min), similar to the analysis described in the previous section (Section 3.7.1.8.1).

If deemed appropriate, treatment contrasts for significant orthogonal polynomial effects: linear, quadratic, cubic etc. were assessed.

A Forest plot displays back-transformed treatment comparison estimates and 90% CIs. If deemed appropriate, a display of subgroup analyses for gender, age group and other potential influencing factors was added to the tables and graphs of this analysis.

C-Peptide (Fasted, All Visits)

C-Peptide levels (fasted, month 3 to month 24) were listed by patient and visit and tabulated in a summary table by visit and treatment.

To compare treatment C-peptide (fasted) levels from month 6 to month 24, a mixed model repeated measurement RMANCOVA for logarithmized C-peptide (fasted) values was performed with the interaction term visit*treatment, with sex and age group as fixed categorical effects, and with the respective log baseline value (prior to first treatment, Day 0) as the continuous covariate for the within-subject repeated measurements during follow-up , as previously described.

If deemed appropriate, treatment contrasts for the significant orthogonal polynomial effects linear, quadratic, cubic etc. were assessed.

A Forest plot displays back-transformed treatment comparison estimates and 90% CIs. If deemed appropriate, a display of subgroup analyses for gender, age group and other potential influencing factors was added to this analysis' tables and graph.

C-Peptide (Glucagon Test, All Visits)

The AUC of C-Peptide levels (glucagon test, 0-6 min, month 3 to month 24 visits) was listed by patient and visit and tabulated in a summary table by visit and treatment.

For the AUC of C-peptide levels during the glucagon test (0-6 min), all RMANCOVAs previously described above were performed for logarithmized AUC C-peptide (Glucagon, (0-6 min) values, with sex, age group and the treatment *visit interaction as fixed categorical effects and the respective log baseline AUC value (prior to first treatment, Day 0) as a covariate, and within-subject repeated measurements during follow-up analyzed in a manner similar to that described for the MMTT test.

If deemed appropriate, treatment contrasts for significant orthogonal polynomial effects (linear, quadratic, cubic etc.) were assessed here as well.

A Forest plot displays back-transformed treatment comparison estimates and 90% CIs. If deemed appropriate, a display of subgroup analyses for gender, age group and other potential influencing factors was added to this analysis tables and graph.

Results for C-Peptide levels (glucagon test, 0 min and 6 min, month 3 to month 24 visits) were descriptively summarized, and treatments were also compared for each timepoint by visit with a RMANCOVA.

HbA1c (All Visits)

HbA1c values (month 3 to month 24 visits) were listed by patient and visit and tabulated in a summary table by visit and treatment.

For HbA1c values, a RMANCOVA was performed based on logarithmized values using treatment*visit interaction, sex and age group as class variables and the respective log baseline value (prior to first treatment, Day 0) as a continuous covariate for the within-subject repeated measurements of all follow up visits. A comparison of differences among treatments was performed with LSmeans and their 90% CIs and was provided on an exploratory basis after retransformation.

A Forest plot displays treatment ratio estimates and 90% CIs. If deemed appropriate, a display of subgroup analyses for gender, age group and other influence factors possible was added to this analysis tables and graph.

A mean concentration time plot with SD interval and a geometric mean plot with 90% CIs was also provided.

Glucose (All Visits)

Glucose levels (month 3 to month 24 visits) were listed by patient and visit and tabulated in a summary table by visit and treatment.

For glucose values, a RMANCOVA was performed as described above based on logarithmized values on an exploratory basis.

A Forest plot displays treatment ratio estimates and 90% CIs. If deemed appropriate, a display of subgroup analyses for gender, age group and other influence factors possible was added to this analysis' tables and graph.

A mean concentration time plot with SD interval and a geometric mean plot with 90% CI were also provided for the glucose levels.

Daily Insulin Dose per kg Body Weight (DDI, All Visits)

DDI values (month 3 to month 24 visits) were listed by patient and visit and tabulated in a summary table by visit and treatment.

For the DDI, a RMANCOVA was performed in a manner similar to the methods described above and in an exploratory basis. This analysis was performed on original untransformed values and using the original baseline value as a covariate.

The proportion of subjects with DDI ≤ 0.5 UI/kg b.w. was displayed in a frequency table by visit and treatment.

A Forest plot displays treatment difference estimates and 90% CIs. If deemed appropriate, a display of subgroup analyses for gender, age group and other influence factors possible was added to this analysis' tables and graph.

Patients in Remission

Remission (defined as DDI <0.5 UI/kg/day and HbA1c <6.5%) assessed for each of the follow-up visits was listed by patient and visit and analyzed using the exact permutation test on the treatment x remission (y/n) contingency table to obtain p-values for all three treatment comparisons.

Insulin-Independent Patients

Insulin Independent Patients (DDI < 0.5 IU/kg) for each of the follow up visits was listed by patient and visit and analyzed using the exact permutation test on the treatment x insulin independence (y/n) contingency table to obtain p-values for all three treatment comparisons.

A survival analysis table for insulin independence is provided for data starting from 3 months.

A Kaplan-Meier plot of the proportions of patients in whom insulin independence was maintained is displayed with treatments overlaid for the corresponding weeks starting from first follow-up (month 3) onwards.

3.7.1.9. Analysis of safety variables

The following categories of safety variables will be analyzed:

- AEs and SAEs
- Clinical laboratory assessments, if collected (blood chemistry, hematology, coagulation and urine analysis)

All safety data will be provided in by-patient listings. The safety population will be used for the analysis of safety variables.

3.7.1.10. Adverse Events

All AEs and ADRs were coded using MedDRA and listed by System Organ Class (SOC) and Preferred Term (PT). All AEs for each patient, including multiple occurrences of the same event, were listed providing verbatim terms, SOC, PT, treatment, severity, seriousness, relation to study treatment, action taken and outcome (where information is available). Details about SAEs and those leading to death or withdrawal were listed.

In the tabulations, the number of patients reporting any AEs as well as the number of AEs were counted separately. Any patients reporting the same event more than once were counted only once within SOC and PT categories (collapsing multiple occurrences of the same event within patient to one event of the maximum severity grade and causality).

A summary table was provided by treatment showing numbers and percentages of patients as well as numbers and percentages of events, and was categorized as follows:

- any AE
- any AE related to the treatment
- any AE by severity (any AE with missing severity will be analyzed as severe)
- any SAE by seriousness (any AE with missing seriousness will be analyzed as serious)
- any AE leading to withdrawal

In addition to the summary table, numbers and percentages of patients as well as numbers and percentages of events were presented by treatment, SOC and PT as follows:

- AEs
- AEs by Relationship
- AEs by Severity
- SAEs
- AEs Leading to Withdrawal

3.7.1.11. Clinical Laboratory Assessments

All laboratory variables (lab values including blood test+CRP+urine test), were listed by patient and time point. Values out of the normal range were flagged.

All parameters with sufficient number of subjects included (>3) were tabulated with descriptive statistics.

3.7.1.12. Immunophenotype

Investigations for immunophenotype like percentage of B lymphocytes and Tregs were listed and tabulated with descriptive statistics by visit and treatment.

In case data on HLA susceptibility and predisposition (first degree relative with T1D) are available these values will also be displayed.

A mean concentration time plot with SD interval was provided.

3.7.1.13. Autoantibodies

Autoantibodies were listed and tabulated by visit and treatment, if available.

The influence of Autoantibodies on main objectives were investigated and, if deemed appropriate, corresponding subgroup analyses were performed and displayed for the main objectives.

A mean concentration time plot with SD interval was provided.

3.7.1.14. Cytokines

Cytokine assessments will be analyzed and reported separately.

3.7.1.15. Molecular Tests

Molecular tests will be analyzed and reported separately.

3.7.1.16. Quality of Life

The quality of life assessment will be analyzed and reported separately.

3.7.2. Changes in Planned Analyses Prior to Unblinding or Database Lock

There were no changes in the planned analyses for the study.

3.7.3. Changes Following Study Unblinding/Database Lock and Post-hoc Analyses

Study Data Tabulation Model (SDTM) Database Lock was done after sponsor approval on 06-Aug-2020. After Tables, Listings, Figures (TLF) review, sponsor requested further changes of the SDTM data by mail on 10-Aug-2020. The following updates for the SDTM laboratory domain (LB) dataset have been done as requested:

- For patient Tv09 the Glucose value at month 24 is given as “>2000.00” in error. This value had been deleted as requested by sponsor.

- For patient Tv18 the Insulin Autoantibody value at month 3 is given as “148” in error. Value had been changed to “6.6” instead as requested by sponsor.

- For patient TvK11 the Insulin Autoantibody value at month 12 is given as “102” in error. Value had been changed to “0.87” instead as requested by sponsor.

After all changes were done, the SDTM database was locked again and sent to the sponsor for new approval.

Changes made after the final SAP and before unblinding or database lock (for open label studies) are described here.

- Note to file (NTF) BI 02 is an AE SAP deviation that describes one difference from the final SAP (2.0) dated 05 August 2020:
    - In the final SAP it is stated that any patients reporting the same event more than once will be counted only once within SOC and PT categories (collapsing multiple occurrences of the same event within the patient to one event of the maximum severity grade and causality).
    - The following deviation was implemented: Subjects will be counted once in all categories as present and not only the worse-case per SOC and PT. Events will be counted as occurred.
- NTF BI 03 is a final SAP v 2.0 clarification. In the study POT_708_TregVac / TregVac2, we differ from the final SAP v 2.0 dated 05 August 2020, in additional to the specified level of insulin independence level (DDI < 0.5

U/kg/day), we also constructed exploratory display tables, figures, and listing for the insulin independence level zero (DDI – 0 U/kg/day).

4. STUDY PARTICIPANTS

4.1. Disposition of Participants

4.1.1. Treatment allocation

The first patient first visit (FPFV) was on 15 JUN 2015. The last patient last visit (LPLV) was 18 Oct 2019 (Listing 16.2.1.1). The cut-off date for this report is 6-Aug-2020.

Thirty-six (36) patients aged 9-16 years were screened in the study (Table 4-1, Table 14.1.1). Patient TV07 was stopped between first donation and dosing due to false-positive Quantiferon results. Later, TV07 was rescreened and became patient TVK03 (Listing 16.2.1.4).

Table 4-1 shows the disposition of the patients. Eleven (11) screened patients did not receive any treatment and were included in the control group. All remaining screened patients were randomly allocated into one of the two treatment groups (Table 14.1.1, Listing 16.2.1.1). Specifically, 13 patients were assigned to the group Tregs + placebo, and 12 patients were assigned to the group Tregs + anti-CD20 antibody rituximab.

All 12 of the patients in the Tregs+anti-CD20 antibody rituximab group completed the study. In the Tregs+placebo group, patients TV01 and TV15 were discontinued. Patient TV01 received only the first dose of Tregs and the first dose of placebo, while patient TV15 received both Tregs doses and all four doses of placebo (Listing 16.2.1.1, Listing 16.2.5.3). In the control group, one patient, TVK03, was discontinued. In all three patients, the reason for discontinuation was unknown (Listing 16.2.1.2).

Table 4-1: Patient Disposition – Frequency Table (All Screened Patients)

	Tregs + Placebo (N=13)	Tregs + anti-CD20 rituximab (N=12)	Control (N=11)	Total (N=36) (%)
Screened				36 (100)
Screen failures				1
Randomized	13	12	11	36
Treated	13	12	11	36
Completed study	11 (84.6%)	12	10 (90.9%)	33
Discontinued study	2 (15.4%)	0	1 (9.1%)	3 (8.3%)
Unknown	2 (15.4%)	0	1 (9.1%)	3 (8.3%)

Abbreviations: Tregs: T regulatory cells, N: number of treated patients
Note(s): Percentages are based on the number of randomized patients within each treatment or overall, as appropriate.
Patient TV07 was stopped between first donation and dosing due to false-positive Quantiferon results. Later, TV07 was rescreened and became Patient TVK03.
% - (n/N)*100 where N is the number of treated patients.
In the control group patients had been recruited from the randomized treatment groups (Tregs and Tregs+anti-CD20 Rituximab) if patients were not applicable for active treatment. The randomization date of the control group corresponds to the original assignment.
Source: Table 14.1.1

The disposition of the patients according to treatment group allocated at the time of randomization is summarized in Table 4-2.

Table 4-2: Treatment Group Allocation – Randomization Set

Treatment	N	Subject ID
Tregs + placebo	13	TV01 TV02 TV05 TV06 TV08 TV09 TV13 TV15 TV16 TV19 TV20 TV22 TV25
Tregs + anti-CD20 rituximab	12	TV03 TV04 TV10 TV11 TV12 TV14 TV17 TV18 TV21 TV23 TV24 TV26
Control	11	TVK01 TVK02 TVK03 TVK04 TVK05 TVK06 TVK07 TVK08 TVK09 TVK10 TVK11

Abbreviations: Tregs: T regulatory cells, N: number of treated patients, ID: Identification
Source: Listing 16.2.1.1

4.2. Protocol Deviations

All protocol deviations are listed by patient in Listing 16.2.2.1. Only one protocol deviation was considered important, and the patient was therefore excluded from the PP set, as reported in Listing 16.2.3.1.

Table 4-3: Participants Excluded from PP Population

Patient number	Reason for exclusion
TV02	Diagnosis of autoimmune-mediated multiorgan endocrine insufficiency

Abbreviations: PP: Per-Protocol
Source: Listing 16.2.2.1, Listing 16.2.3.1

4.3. Populations Analyzed

The different analysis sets were defined in Section REF _Ref51927411 \r \h 3.7.1.2. All 36 patients allocated to treatment received treatment and were included in the safety (SAF) and intention to treat (ITT) sets. Patient TV02 had an important protocol deviation and was excluded from the per protocol (PP) set (Section 4.2). The number of patients included in each analysis population is provided in Table 4-4.

A listing of patients excluded from the different analysis sets and the respective reasons is shown in Listing 16.2.3.1.

Table 4-4: Datasets Analyzed

	Tregs + Placebo (N=13)	Tregs + anti-CD20 rituximab (N=12)	Control N=11	Total (N=36) (%)
Safety set	13	12	11	36 (100)
ITT set	13	12	11	36
PP set	12	12	11	35

Abbreviations: N: number of treated patients, ITT: intention-to-treat, PP: per protocol, Tregs: T regulatory cells
Source: Table 14.1.1, Listing 16.2.1.1

4.4. Demographic and Other Baseline Characteristics

4.4.1. Demography

Summaries of descriptive statistics for demographics and baseline characteristics of the ITT set are presented in Table 14.1.3.1 and Table 4-5. Individual listings of demographic data are shown by patient in Listing 16.2.4.1. Summary statistics for the PP set are shown in Table 14.1.3.2.

Table 4-5: Demographic Data – Summary Table (ITT set)

Parameter			Tregs + anti-CD20 rituximab (N=12)	Tregs + Placebo (N=13)	Control N=11	Total (N=36)
Gender, n (%)	Male	n (%)	5 (41.7)	7 (53.8)	5 (45.5)	17 (47.2)
	Female	n (%)	7 (58.3)	6 (46.2)	6 (54.5)	19 (52.8)
Race	White Caucasian	n (%)	12 (100)	13 (100)	11 (100)	36 (100)
Age (years)		n	12	13	11	36
		Mean	12.9	13.3	12.1	12.8
		SD	1.16	1.49	2.17	1.67
Weight (kg)		n	12	13	11	36
		Mean	45.50	51.85	46.26	48.03
		SD	8.916	9.296	10.461	9.719
Height (cm)		n	12	13	11	36
		Mean	157.7	161.8	157.1	159.0
		SD	9.69	8.31	11.79	9.87
BMI (kg/m^2)		n	12	13	11	36
		Mean	18.109	19.571	18.445	18.740
		SD	1.750	1.781	1.426	1.747

Abbreviations: Tregs: T regulatory cells, n: number of measurements included in the analysis, N: number of treated participants, BMI: body mass index, SD: standard deviation, ITT: intention-to-treat, kg: kilogram, cm: centimeter, m: meter
Note(s): All variables are assessed at Screening; % - (n/N)*100 where N is the number of treated patients.
Source: Table 14.1.3.1, Listing 16.2.4.1

4.4.2. Baseline disease characteristics

Descriptive statistics for number of months since diagnosis by treatment group are presented by patient in Listing 16.2.4.2 and as summary statistics in Table 14.1.4.1 and Table 4-6. By-patient listings for the following laboratory parameters collected at baseline are also provided:

Childbearing potential (Listing 16.2.8.2), where applicable.

Glycaemia test results (fasting) at baseline, defined as the last value assessed prior to the first drug administration (mg/dL, Listing 16.2.4.3).

Autoantibodies and C-peptide test results (ICA (titer), anti-GAD (IU/mL), IAA (IU/mL), C-peptide (fasted, µg/L) are shown in Listing 16.2.4.3), if available.

Table 4-6: Disease Diagnosis Data – Summary Table by Treatment Group (ITT set)

Demographic Parameter	Statistics	Tregs + anti-CD20 rituximab (N=12)	Tregs + Placebo (N=13)	Control (N=11)	Total (N=36)
Months since diagnosis	n	12	13	11	36
	Mean	6.0	6.5	5.0	5.9
	SD	4.20	4.20	3.16	3.85
	Minimum	2	2	3	2
	Median	5.5	6.0	4.0	5.0
	Maximum	17	18	14	18

Abbreviations: Tregs: T regulatory cells, ITT: intention-to-treat, N: number of treated patients, n: number included in the analysis, SD: standard deviation
Note(s): All variables were assessed at screening
Source: Table 14.1.4.1

4.5. Prior, Concomitant, Post-intervention Therapy

Previous and concomitant medication were not specifically collected during the trial and only generally mentioned when a specific treatment given for AE handling. These cases are listed in Listing 16.2.9.1 and, for concomitant medications, also summarized with descriptive statistics and by number and percentage of patients by treatment (Appendix 14, Table 14.1.6.1). Previous and concomitant medications were coded according to the WHO ATC 2020 classification system, if applicable. All listings and tables displaying terms of coding dictionaries include a footnote presenting the version of the dictionary used.

4.6. Exposure and Study Intervention Compliance

4.6.1. Exposure

A by-patient listing of exposure data is provided for Tregs and rituximab/placebo in Listing 16.2.5.1 and Listing 16.2.5.3. These variables are summarized with descriptive statistics for IMP intake: number of injections of Tregs received, and number of injections of rituximab/placebo received (Table 14.1.5.1 and Table 4-7). The percent of scheduled doses of Tregs or rituximab/placebo received for each patient is defined as:

[(Number of IV injections Received) / (Expected Number of IV injections)] *100.

Table 4-7: Extent of Exposure (Tregs) – Summary Table (ITT Set)

	Tregs + anti-CD20 rituximab (N=12)	Tregs + Placebo (N=13)	Total (N=25)
Total number of Treg doses (injections)			
n (%)	12 (100)	13 (100)	25 (69.4)
Mean	2.0	1.9	2.0
SD	0	0.28	0.20
Minimum	2	1	1
Median	2.0	2.0	2.0
Maximum	2	2	2
Total number of Rituximab/Placebo doses (injections)			
n (%)	12 (`00)	13 (100)	25 (100)
Mean	4	3.9	3.9
SD	0.00	0.83	0.60
Minimum	4	1	1
Median	4	4	4
Maximum	4	4	4

Abbreviations: Tregs: T regulatory cells, ITT: intention-to-treat, N: number of treated patients, n: number included in the analysis, SD: standard deviation
Source: Table 14.1.5.1, Listing 16.2.5.1, Listing 16.2.5.3

5. EVALUATION OF RESPONSE TO STUDY INTERVENTION

5.1. Efficacy

The results presented in the following sections are based on the ITT population. The results for the PP population were similar due to the similarity of the two populations: the PP population included all patients of the ITT population except 1 patient (see Section 4.3). Tables and figures based on the data of the PP population are presented in Appendix 14, data listings for individual patients in Appendix 16.

5.1.1. Analysis of primary endpoints

5.1.1.1. AUC of C-peptide (MMTT, 0-240 min) at 24 m (week 104)

The geometric mean ratios of Tregs+anti-CD20 antibody rituximab/control and Tregs/control were obtained from the point estimates and confidence limits of analysis of covariance (ANCOVA) of the logarithmized AUC of C-peptide levels (MMTT, 0-240 min) at 24 months. The results showed that both Tregs + anti-CD20 antibody rituximab (treatment ratio 1.770, 90% CI 1.018 - 3.078) and Tregs (1.893, 90% CI 1.062 – 3.372) was statistically significantly superior to the control group, with both CIs completely above unity. However, in the subsequent non-inferiority comparison of Tregs/Tregs + anti-CD20 rituximab, the CI was not completely above the non-inferiority margin, indicating that the therapeutic equivalence (non-inferiority) of Tregs alone vs. the combined therapy was not supported (1.069, 90% CI 0.601 - 1.902) (Table 5-1, Figure 5-1). Hence, while both treatments performed better than the control, Tregs alone did not outperform Tregs + anti-CD20 antibody rituximab.

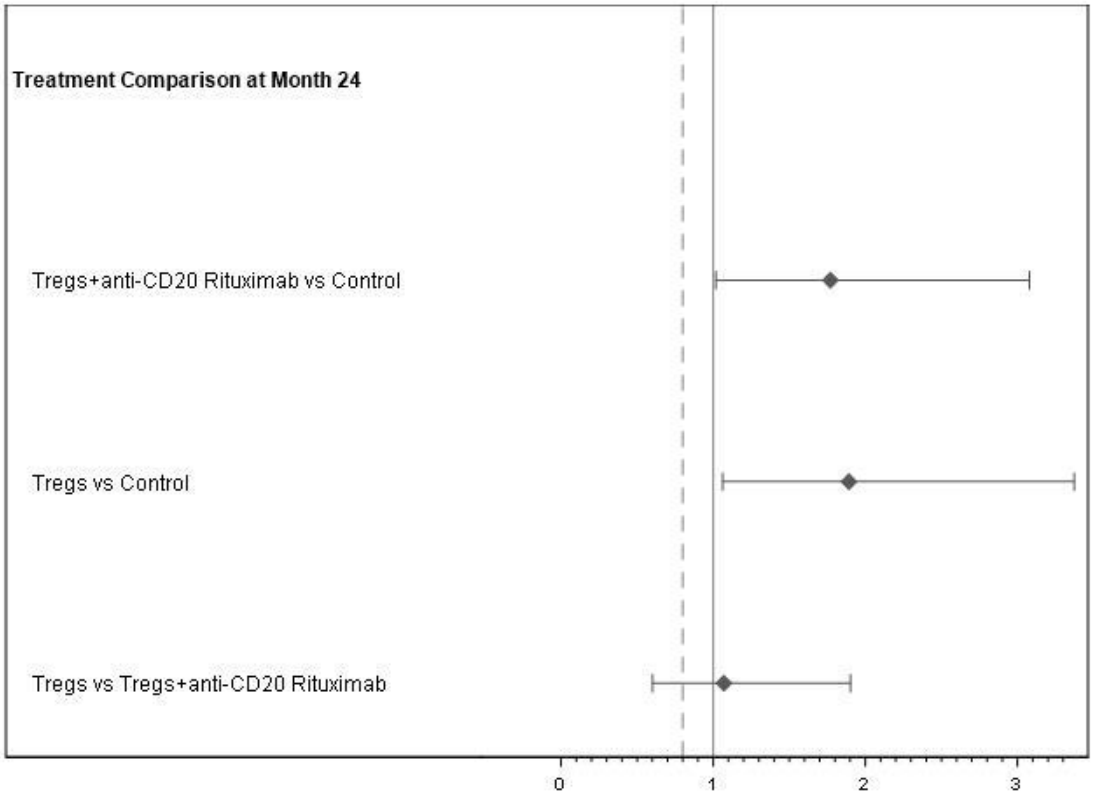
The AUC of C-peptide levels (MMTT, 0-240 min) obtained at 24 months are listed by patient and visit in Listing 16.2.6.1. Descriptive statistics for the AUC values of C-peptide levels and changes from baseline (Day 0) are provided for the treatment groups (Table 14.2.1.1.1 and, for the two superiority comparisons, Table 14.2.1.1.2). ANCOVA results for the AUC of C-peptide (MMTT, 0-240 min) at the 24-month visit is shown in Tables 14.2.1.1.3 and 14.2.1.1.4. Treatment comparisons by geometric mean ratios (point estimates) and their 90% CIs are displayed in Forest Plots and Figures 14.2.1.1.9 and 14.2.1.1.10.

Table 5-1: AUC of C-Peptide (MMTT, 0-240 min, 24m Visit) – Comparison of Treatment Groups (ITT Set)

Visit	Comparison	N	Geometric mean		Treatment ratio	90% CI*
			Test	Reference		
24-m	Tregs+anti-CD20 Rituximab vs Control	32	4.441	2.509	1.770	1.018 - 3.078*
	Tregs vs Control	32	4.750	2.509	1.893	1.062 - 3.372*
	Tregs vs Tregs+anti-CD20 Rituximab	32	4.750	4.441	1.069	0.601 - 1.902

Abbreviations: Tregs: T regulatory cells, N: number of treated patients, min: minutes, m: month; MMTT: mixed meal tolerance test, ITT: intention-to-treat, m: month
Note(s): *indicates a significant difference (these values also boxed)
Results of analysis of covariance (ANCOVA) of logarithmized AUC of C-peptide levels (MMTT, 0-240 min) at 24 m, using the logarithmized AUC of C-peptide levels (MMTT, 0-240 min) at baseline (Day 0) as continuous covariate and age group (either < = 12 years or >12 years), gender and treatment as fixed categorical effects.
Source: Table 14.2.1.1.3, Listing 16.2.6.1

Figure 5-1: Forest Plot (ANCOVA) of Geometric Mean Ratios of C-peptide AUC (MMTT, 0-240 min) (ITT Set)



Abbreviations: Tregs: T regulatory cells, ANCOVA: analysis of covariance, AUC: area under the curve, MMTT: mixed meal tolerance test, ITT: intention-to-treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented
Source: Figure 14.2.1.1.9

5.1.1.2. C-peptide (Glucagon Test, 0 and 6 min) at 24 months

Geometric mean ratios for AUC (0-6 min) of C-peptide levels across the two measurement times of the glucagon test at 24 months were obtained using a statistical approach similar to that described in Section 5.1.1.1. The results showed that while neither group was superior to the control group, Tregs+anti-CD20 antibody rituximab was nearly so (1.704, 90% CI 0.989-2.935), while Tregs was not (0.908, 90% CI 0.514-1.604) (Table 5-2**Błąd! Nie można odnaleźć źródła odwołania.**, Figure 5-2). Although the Tregs+anti-CD20 antibody rituximab group appeared to perform better than Tregs against the control, neither was statistically significantly better than the control. However, in the comparison of the two active treatments, Tregs alone was inferior to the combination therapy (0.533, 90% CI 0.305-0.932).

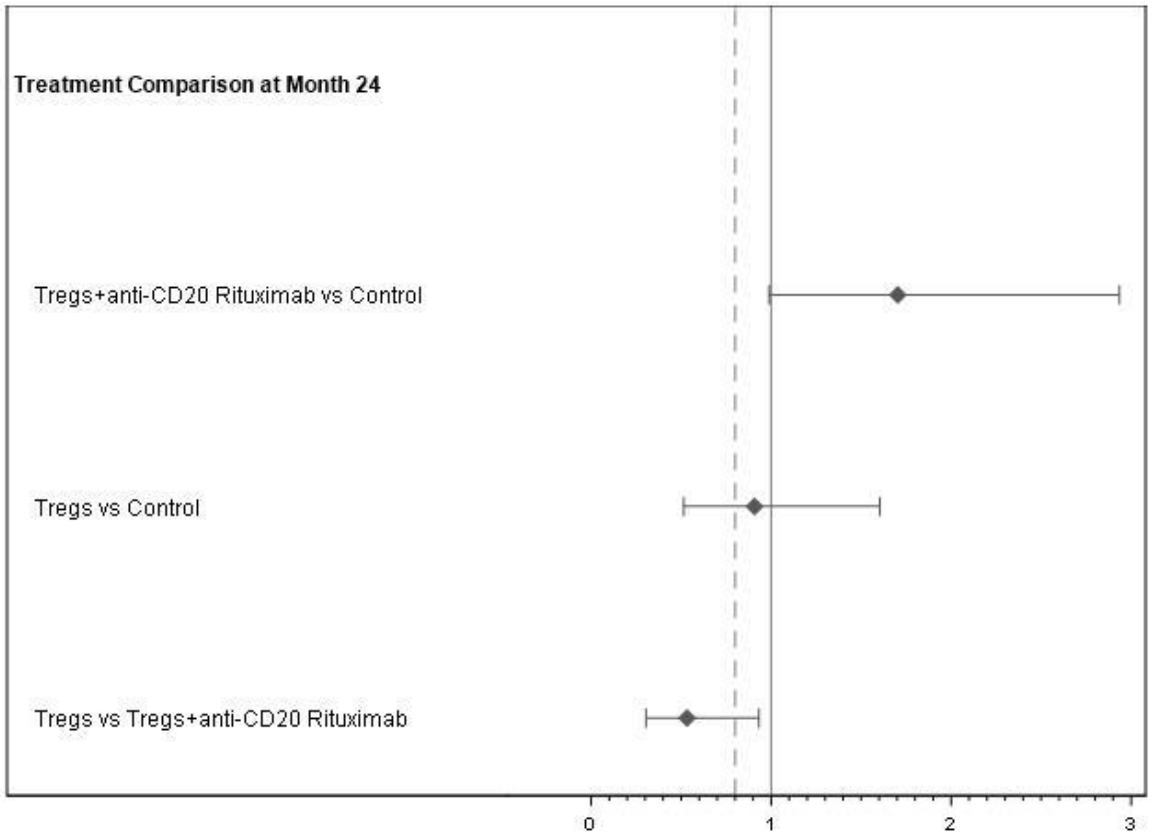
Summary statistics for AUC of C peptide (Glucagon test, 0-6 min) at the 24-month visit are shown as summary tables in Tables 14.2.1.2.1 and 14.2.1.2.2. ANCOVA of logarithmized values of AUC of C peptide (Glucagon Test, 0-6 min) at 24 months are shown in Tables 14.2.1.2.3 and 14.2.1.2.4. Forest Plots showing ANCOVA of C peptide AUC (Glucagon test, 0-6 min) are shown for geometric mean ratios and 90% CIs in and Figure 14.2.1.2.9 and 14.2.1.2.10.

Table 5-2: AUC of C-Peptide (Glucagon Test, 0-6 min, 24-m Visit) – Comparison of Treatment Groups (ITT Set)

Visit	Comparison	N	Geometric mean		Treatment ratio	90% CI
			Test	Reference		
24-m	Tregs+anti-CD20 Rituximab vs Control	32	0.085	0.049	1.704	0.989-2.935
	Tregs vs Control	32	0.045	0.049	0.908	0.514-1.604
	Tregs vs Tregs+anti-CD20 Rituximab	32	0.045	0.084	0.533	0.305-0.932*

Abbreviations: Tregs: T regulatory cells, N: number of treated patients, min: minutes, m: month; ITT: intention-to-treat
Results of analysis of covariance (ANCOVA) of logarithmized AUC of C-peptide levels (Glucagon Test, 0-6 min) at 24m, using the logarithmized AUC of C-peptide levels (Glucagon Test, 0-6 min) at baseline (Day 0) as continuous covariate and age group (either <=12years or >12 years), gender and treatment as fixed categorical effects.
Source: Table 14.2.1.2.3, Listing 16.2.6.5

Figure 5-2: Forest Plot (ANCOVA) of Geometric Mean Ratios of C-peptide AUC (Glucagon Test, 0-6 min) (ITT Set)



Abbreviations: Tregs: T regulatory cells, ANCOVA: analysis of covariance, AUC: area under the curve, MMTT: mixed meal tolerance test, ITT: intention-to-treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented
Source: Figure 14.2.1.2.9

Geometric mean ratios for C-peptide levels (glucagon test, 0 and 6 min) at 24 months were also obtained using a statistical approach similar to that used for the analysis of AUC in Section 5.1.1.1. The results showed that neither group was superior to the control group at either timepoint (at 0 min: 1.497, 90% CI 0.892-2.513 and 1.009, 90% CI 0.6091.673, respectively; and at 6 min: 1.301, 90% CI 0.680-2.490 and 0.791, 90% CI 0.420-01.490,

respectively) (Table 5-3, Figure 5-3). Again, at both timepoints, the Tregs+anti-CD20 antibody rituximab group did relatively better against the control than the Tregs group, but none of the comparisons was statistically significant, and the result of the comparison of the two treatment groups was inconclusive.

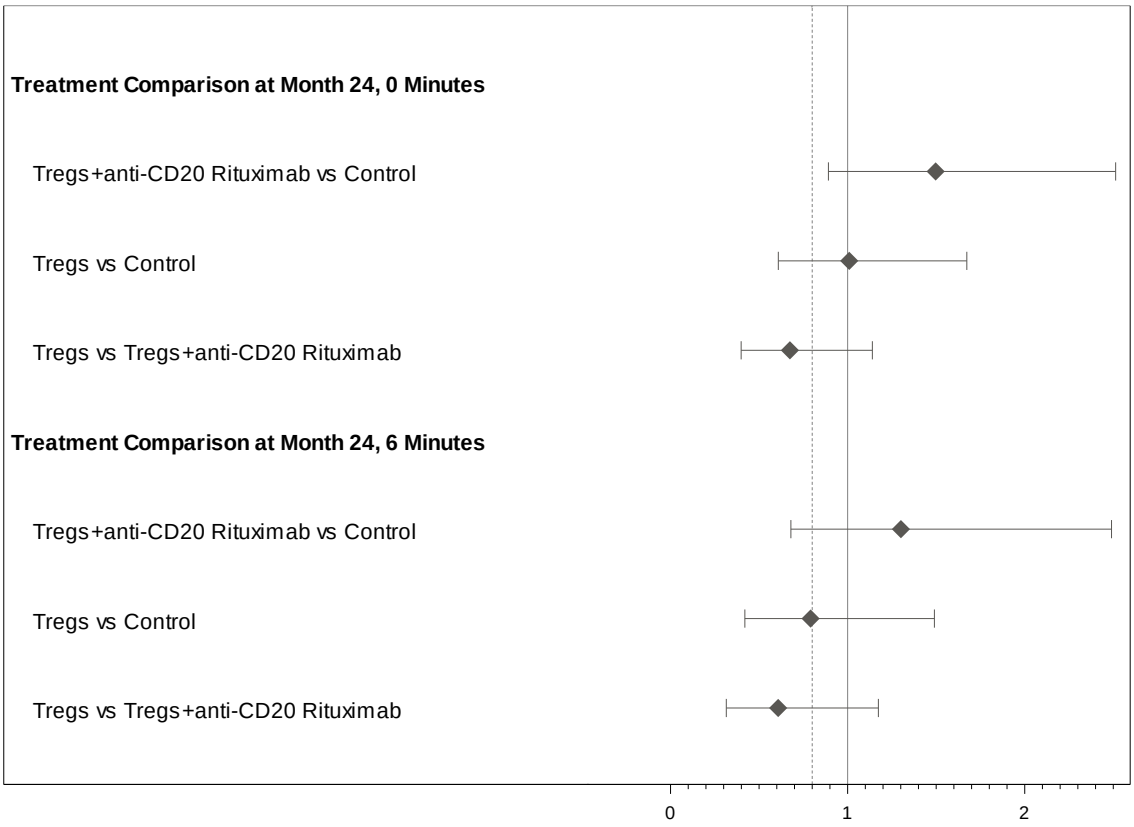
A by-patient listing of C-peptide levels (Glucagon test) is shown in Listing 16.2.6.11. Summary statistics for C-peptide concentration (Glucagon Test, 0 and 6 in) at the 24-month visit are summarized in Tables 14.2.1.7.1 and 14.2.1.7.2. ANCOVA of logarithmized values of C peptide concentration (Glucagon Test, 0 and 6 min) at the 24-month visit are shown in Tables 14.2.1.7.3 and 14.2.1.7.4. Forest plots of ANCOVA of geometric mean ratios of C-peptide levels (Glucagon test) are shown in Figure 14.2.1.7.9 and 14.2.1.7.10.

Table 5-3: C-Peptide (Glucagon Test, 24-m Visit) – Comparison of Treatment Groups (ITT Set)

Visit	Timepoint	Comparison	Geometric mean		Treatment ratio	90% Confidence interval
			Test	Reference		
24-m	**0 min**	Tregs+anti-CD20 Rituximab vs Control	0.514	0.343	1.497	0.892 - 2.513
		Tregs vs Control	0.346	0.343	1.009	0.609 - 1.673
		Tregs vs Tregs+anti-CD20 Rituximab	0.346	0.514	0.674	0.399 - 1.140
	6 min	Tregs+anti-CD20 Rituximab vs Control	0.932	0.716	1.301	0.680 - 2.490
		Tregs vs Control	0.566	0.716	0.791	0.420 - 1.490
		Tregs vs Tregs+anti-CD20 Rituximab	0.566	0.932	0.608	0.315 - 1.174

Abbreviations: Tregs: T regulatory cells, N: number of treated patients, min: minutes, m: month; ITT: intention-to-treat
Results of analysis of covariance (ANCOVA) of logarithmized C-peptide levels (Glucagon Test) at 0min and 6min, using the logarithmized C-peptide levels (Glucagon Test) at baseline (Day 0, 0 min) as continuous covariate and age group (either <=12years or >12 years), gender and treatment as fixed categorical effects.
Source: Table 14.2.1.7.3, Listing 16.2.6.11

Figure 5-3: Forest Plot (ANCOVA) of Geometric Mean Ratios of C-peptide (Glucagon Test) (ITT Set)



Abbreviations: Tregs: T regulatory cells, ANCOVA: analysis of covariance, AUC: area under the curve, ITT: intention-to-treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented
Source: Figure 14.2.1.7.9

5.1.1.3. C-peptide (MMTT, 0-240 min) at 24 months

Geometric mean ratios for C peptide levels (MMTT, 0-240 min) at 24 months were obtained using a statistical approach similar to that described in Section 5.1.1.1. The results showed that the treatment groups were statistically significantly superior (both 90% CIs completely above unity) to the control group at only one timepoint: 150 minutes (1.877, 90% CI 1.019-3.456 and 2.204, 90% CI 1.181-4.111, respectively). However, in the subsequent non-inferiority comparison of the ratio Tregs/Tregs+anti-CD20 antibody rituximab, the CI was not completely above the non-inferiority margin, showing that the therapeutic equivalence of Tregs alone vs the combined therapy was not supported (Table 5-4, Figure 5-4).

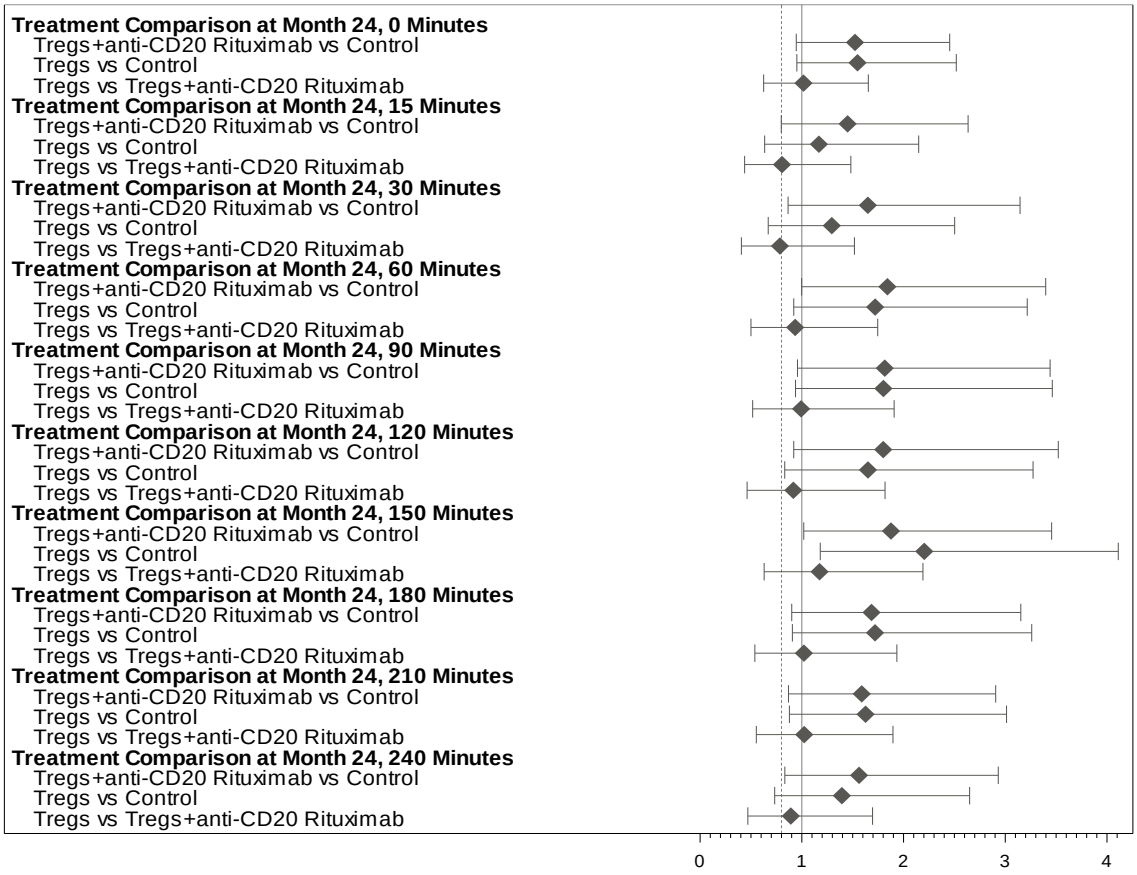
Summary statistics for blood C-peptide levels (MMTT, 0-240 min) at the 24-month visit are shown in Table 14.2.1.3.1 and 14.2.1.3.2. ANCOVA of logarithmized C-peptide levels (MMTT, 0-240 min) are shown in Tables 14.2.1.3.3 and 14.2.1.3.4. Forest plots (ANCOVA, Figures 14.2.1.3.9 and 14.2.1.3.10) show treatment comparisons by geometric mean ratios (point estimates) and their 90% CIs for C-peptide (MMTT, 0-240) at the 24-month visit

Table 5-4: C-Peptide (MMTT, 0-240 min, 24m visit) – Comparison of Treatment Groups (ITT Set)

Timepoint	Comparison	Geometric mean		Treatment ratio	90% Confidence interval
		Test	Reference		
0 min	Tregs+anti-CD20 Rituximab vs Control	0.349	0.229	1.523	0.945 - 2.454
	Tregs vs Control	0.355	0.229	1.548	0.951 - 2.519
	Tregs vs Tregs+anti-CD20 Rituximab	0.355	0.349	1.016	0.624 - 1.654
15 min	Tregs+anti-CD20 Rituximab vs Control	0.538	0.371	1.450	0.798 - 2.635
	Tregs vs Control	0.433	0.371	1.168	0.635 - 2.149
	Tregs vs Tregs+anti-CD20 Rituximab	0.433	0.538	0.805	0.438 - 1.482
30 min	Tregs+anti-CD20 Rituximab vs Control	0.780	0.473	1.649	0.865 - 3.145
	Tregs vs Control	0.612	0.473	1.295	0.670 - 2.503
	Tregs vs Tregs+anti-CD20 Rituximab	0.612	0.780	0.785	0.406 - 1.517
60 min	Tregs+anti-CD20 Rituximab vs Control	1.201	0.652	1.842	0.998 - 3.398
	Tregs vs Control	1.122	0.652	1.721	0.921 - 3.216
	Tregs vs Tregs+anti-CD20 Rituximab	1.122	1.201	0.934	0.500 - 1.746
90 min	Tregs+anti-CD20 Rituximab vs Control	1.342	0.739	1.815	0.957 - 3.441
	Tregs vs Control	1.332	0.739	1.802	0.938 - 3.463
	Tregs vs Tregs+anti-CD20 Rituximab	1.332	1.342	0.993	0.517 - 1.908
120 min	Tregs+anti-CD20 Rituximab vs Control	1.509	0.838	1.800	0.921 - 3.521
	Tregs vs Control	1.383	0.838	1.650	0.832 - 3.273
	Tregs vs Tregs+anti-CD20 Rituximab	1.383	1.509	0.917	0.462 - 1.818
150 min	Tregs+anti-CD20 Rituximab vs Control	1.333	0.710	1.877	1.019 - 3.456*
	Tregs vs Control	1.566	0.710	2.204	1.181 - 4.111*
	Tregs vs Tregs+anti-CD20 Rituximab	1.566	1.333	1.174	0.630 - 2.191
180 min	Tregs+anti-CD20 Rituximab vs Control	1.221	0.724	1.685	0.901 - 3.153
	Tregs vs Control	1.246	0.724	1.720	0.907 - 3.260
	Tregs vs Tregs+anti-CD20 Rituximab	1.246	1.221	1.021	0.538 - 1.934
210 min	Tregs+anti-CD20 Rituximab vs Control	1.054	0.663	1.589	0.869 - 2.906
	Tregs vs Control	1.078	0.663	1.626	0.878 - 3.013
	Tregs vs Tregs+anti-CD20 Rituximab	1.078	1.054	1.024	0.553 - 1.896
240 min	Tregs+anti-CD20 Rituximab vs Control	1.005	0.643	1.563	0.833 - 2.931
	Tregs vs Control	0.896	0.643	1.394	0.733 - 2.649
	Tregs vs Tregs+anti-CD20 Rituximab	0.896	1.005	0.892	0.469 - 1.695

Abbreviations: Tregs: T regulatory cells, MMTT: mixed meal tolerance test, ITT intention-to-treat, min: minute, m: month
Note(s): * indicates a significant difference (these values also boxed)
Results of analysis of covariance (ANCOVA) of logarithmized C-peptide levels (MMTT Test) by time point, using the logarithmized C-peptide levels (MMTT, 0-240min) at baseline (Day 0, 0 min) as continuous covariate and age group (either <=12years or >12 years), gender and treatment as fixed categorical effects.
Source: Table 14.2.1.3.3, Listing 16.2.6.2

Figure 5-4: Forest Plot (ANCOVA) of Geometric Mean Ratios of C-peptide (MMTT, 0-240 min) (ITT Set)



Abbreviations: Tregs: T regulatory cells, ANCOVA: analysis of covariance, MMTT: mixed meal tolerance test, ITT: intention-to-treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented
Source: Figure 14.2.1.3.9

5.1.1.4. C-Peptide (Fasted, 24-month visit)

Geometric mean ratios for fasted C-peptide levels at the 24-month visit were obtained using statistical methods similar to those described in Section 5.1.1.1. The results showed that while the combined treatment was statistically significantly superior to the control (2.268, 90% CI 1.264-4.069), the monotherapy was not (1.253, 90% CI 0.692-2.270). This difference in the results of the comparisons of the two active treatments vs the control group is further underlined by the comparison for non-inferiority of Tregs alone vs. the combined therapy, which pointed towards inferiority of the monotherapy (0.553, 90% CI 0.309-0.989) (Table 5-5, Figure 5-5).

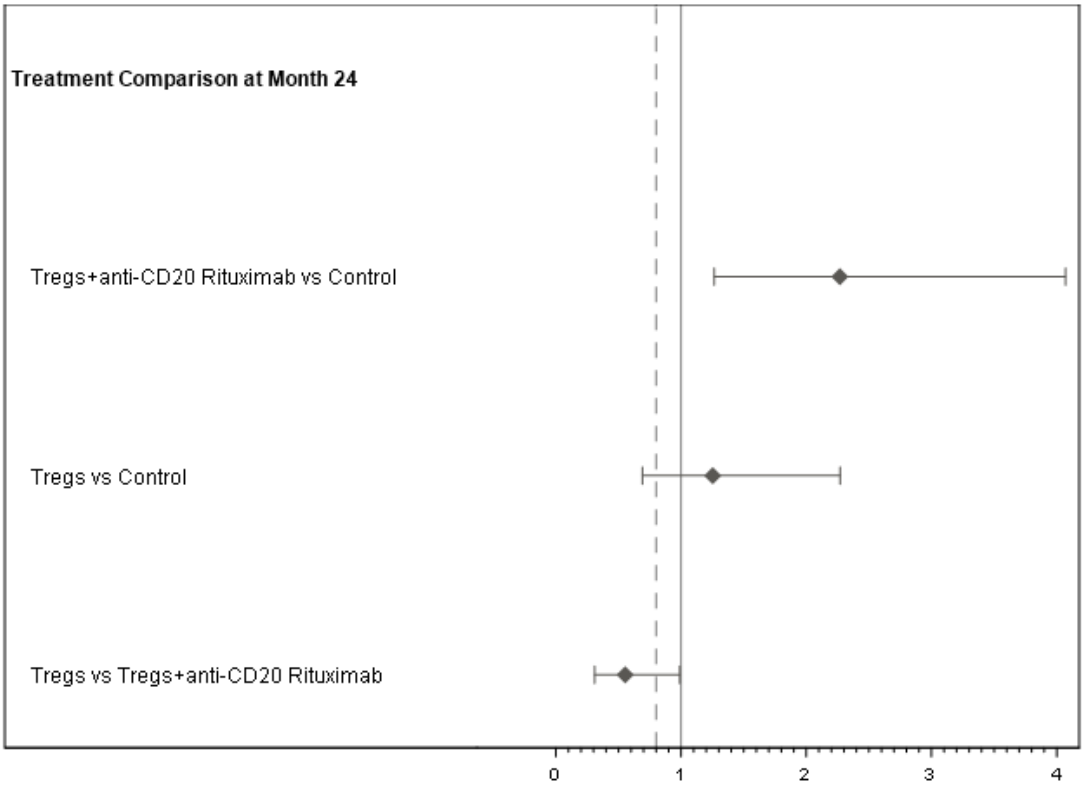
Summary statistics for fasted C peptide levels at the 24-month visit are listed in Listing 16.2.6.3 and shown by patient and visit in Tables 14.2.1.4.1 and 14.2.1.4.2. Treatment comparisons (ANCOVA) are shown by geometric mean ratios (point estimates) and their 90% CIs in Table 14.2.1.4.3 and 14.2.1.4.4 and as Forest Plots in Figures 14.2.1.4.9 and 14.2.1.4.10.

Table 5-5: C-Peptide (Fasted, 24-m Visit) – Comparison of Treatment Groups (ITT Set)

Visit	Comparison	Geometric mean		Treatment ratio	90% Confidence interval
		Test	Reference		
24-m	Tregs+anti-CD20 Rituximab vs Control	0.619	0.273	2.268	1.264 - 4.069*
	Tregs vs Control	0.342	0.273	1.253	0.692 - 2.270
	Tregs vs Tregs+anti-CD20 Rituximab	0.342	0.619	0.553	0.309 - 0.989*

Abbreviations: Tregs: T regulatory cells, m: month, ITT: intention-to-treat, N: number of treated patients
Note(s): N: number of observations used in the analysis. * indicates a significant difference indicates (these values also boxed). Results of analysis of covariance (ANCOVA) of logarithmized fasted C-peptide levels, using the logarithmized fasted C-peptide levels at baseline as continuous covariate and age group (either <=12years or >12 years), gender and treatment as fixed categorical effects.
Source: Table 14.2.1.4.3, Listing 16.2.6.3

Figure 5-5: Forest Plot (ANCOVA) of Geometric Mean Ratios of C-peptide (Fasted) (ITT Set)



Abbreviations: Tregs: T regulatory cells, ANCOVA: analysis of covariance, ITT: intention-to-treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented
Source: Figure 14.2.1.4.9

5.1.1.5. Exogenous Daily Insulin Dose (24-month Visit)

Mean daily insulin use increased in all three groups over the course of the study (Figure 14.2.1.5.5). The point estimates for the treatment differences Tregs+anti-CD20 antibody rituximab vs. control and Tregs vs. control (ANCOVA) showed neither treatment group was superior to the control group at 24 months (treatment difference, -0.126, 90% CI -0.302-0.051

and -0.172m 90% CI -0.357-0.012, respectively). Furthermore, there was no statistically significant difference between the two treatment groups (-0.047, 90% CI -0.213-0.119) (Table 5-6, Figure 5-6). These results indicate that neither the combination therapy nor the monotherapy significantly affected the daily insulin dose at 24 months.

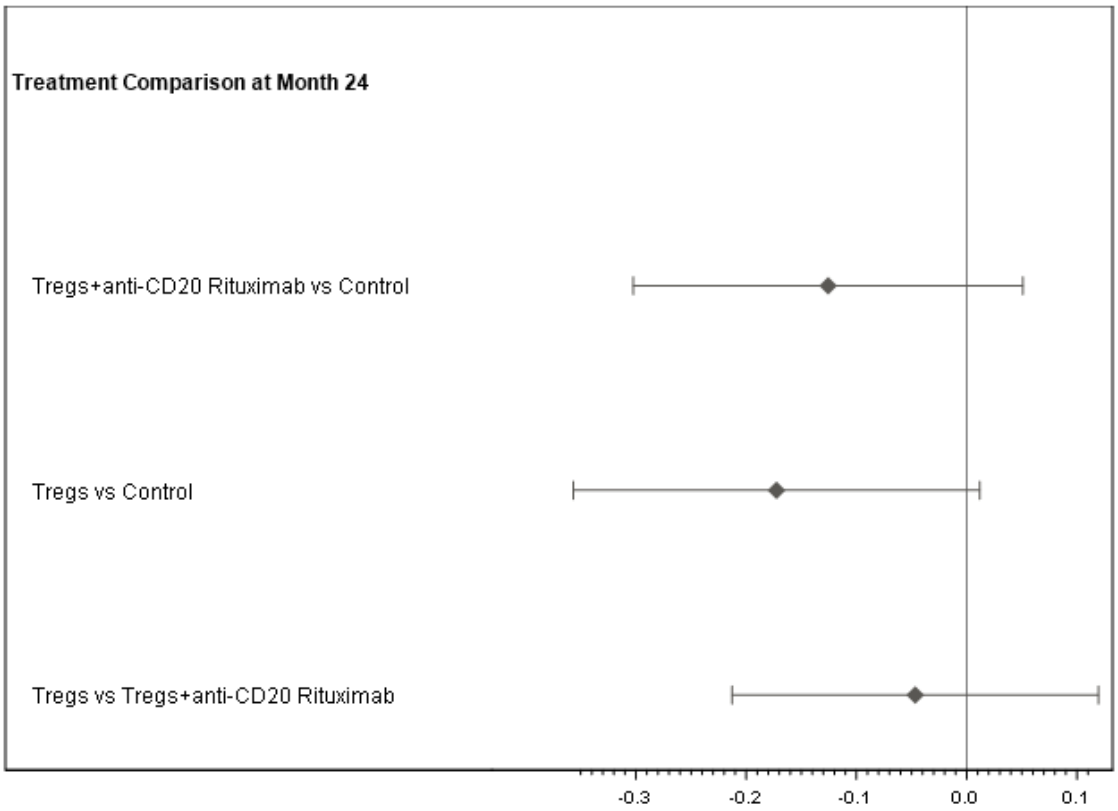
A by-patient listing of daily insulin dose per kg body weight is shown by visit in Listing 16.2.6.8. Summary statistics for the DDI at the 24-month visit are shown in Tables 14.2.1.5.1 and 14.2.1.5.2. ANCOVA results for treatment comparisons of DDI at the 24-month visit are shown by geometric mean ratios (point estimates) with 90% CIs in Table 14.2.1.5.3 and 14.2.1.5.4 and as Forest Plots in Figure 14.2.1.5.9 and 14.2.1.5.10.

Table 5-6: Daily Insulin Dose per kg Body Weight (24m Visit) – Comparison of Treatment Groups (ITT Set)

Visit	Comparison	Test	Mean		Treatment Difference	90% Confidence interval
			Reference			
24-m	Tregs+anti-CD20 Rituximab vs Control	0.605	0.730		-0.126	-0.302 - 0.051
	Tregs vs Control	0.558	0.730		-0.172	-0.357 - 0.012
	Tregs vs Tregs+anti-CD20 Rituximab	0.558	0.605		-0.047	-0.213 - 0.119

Abbreviations: Tregs: T regulatory cells, m: month, ITT: intention-to-treat, N: number of observations used in the analysis, kg: kilogram
Note(s): Results of analysis of covariance (ANCOVA) of daily dose of insulin by body weight, using the daily dose of insulin by body weight at baseline as continuous covariate and age group (either <=12years or >12 years), gender and treatment as fixed categorical effects.
Source: Table 14.2.1.5.3, Listing 16.2.6.8

Figure 5-6: Forest Plot (ANCOVA) of Arithmetic Mean Differences of Daily Insulin Dose per kg body weight (ITT Set)



Abbreviations: Tregs: T regulatory cells, ANCOVA: analysis of covariance, ITT: intention-to-treat, kg: kilogram
Note(s): Differences of the treatment means and 90 % confidence intervals are presented.
Source: Figure 14.2.1.5.9

5.1.1.6. Remission (12 months [week 52] and 24 months [week 104] Visit)

Remission (defined as DDI <0.5 UI/kg/day and HbA1c <6.5%) was assessed with the exact permutation test (‘treatment*remission contingency table). The results showed that there was no statistically significant difference in the proportion of patients in remission between the control group and either the Tregs+anti-CD20 antibody rituximab group or the Tregs group at 12 months (p=0.1187 and 0.8193, respectively) or 24 months (p=0.2333 and p=0.4581, respectively). There was also no difference between the two treatment groups at 12 or 24 months (p=0.2679 and 0.8137, respectively) (Table 5-6, Table 5-7). However, there was a clear trend in the time-to-event (time to first loss of remission) analysis for the proportion of patients in remission to decrease more slowly in the combination therapy group than in either the monotherapy group or the control group (Table 5-9, Figure 5-7). Note that in this analysis, for patients whose remission status was known, those who entered, left, and re-entered remission were censored for timepoints subsequent to the initial leaving of remission. Patients with unknown remission status were censored from the analysis.

A by-patient listing of patient remission status is shown by visit in Listing 16.2.6.9. Summary statistics for remission after 12 and 24 months are shown in Table 14.2.1.6.1 to Table 14.2.1.6.4. Individual listings are shown in Listing 16.2.6.9. Survival analyses of remission starting from month 3 are shown in Table 14.2.1.6.5 and 14.2.1.6.6. A Kaplan-Meier plot of clinical remission rates is displayed with treatments overlaid by time (weeks) from the 3-month to the 24-month visit (Figure 14.2.1.6.7 and 14.2.1.6.8).

Table 5-7: Remission (12-month Visit) - Contingency Table (ITT Set)

Comparison	Proportion of patients in remission Test [%]	Proportion of patients in remission Reference [%]	p-value
Tregs+anti-CD20 Rituximab vs Control	54.5	20.0	0.1187
Tregs vs Control	30.0	20.0	0.8193
Tregs vs Tregs+anti-CD20 Rituximab	30.0	54.5	0.2679

Abbreviations: Tregs: T regulatory cells, m: month, ITT: intention-to-treat
Note(s): p-value is calculated in a permutation exact test.
Source: Table 14.2.1.6.1, Listing 16.2.6.9

Table 5-8: Remission (24-month Visit) - Contingency Table (ITT Set)

Comparison	Proportion of patients in remission Test [%]	Proportion of patients in remission Reference [%]	p-value
Tregs+anti-CD20 Rituximab vs Control	33.3	10.0	0.2333
Tregs vs Control	27.3	10.0	0.4581
Tregs vs Tregs+anti-CD20 Rituximab	27.3	33.3	0.8137

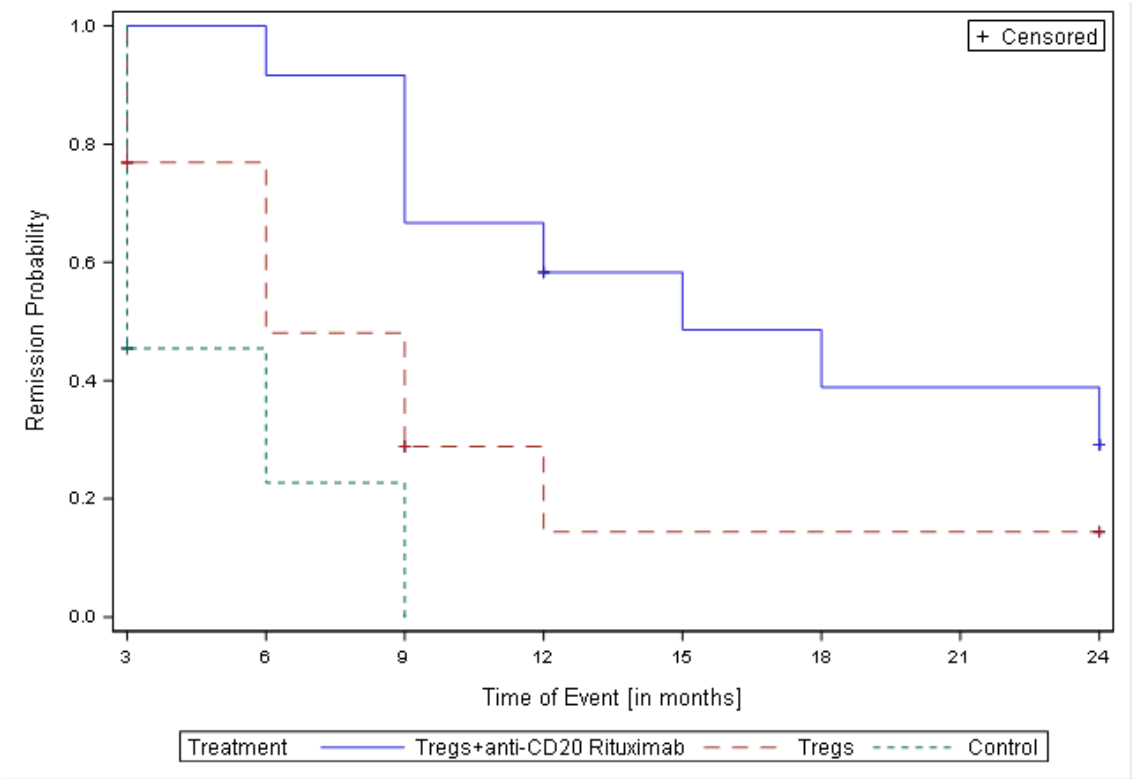
Abbreviations: Tregs: T regulatory cells, m: month, ITT: intention-to-treat
Note(s): p-value is calculated in a permutation exact test.
Source: Table 14.2.1.6.3, Listing 16.2.6.9

Table 5-9: Survival Analysis of Time to First Loss of Remission Starting at the 3m Visit (ITT Set)

Visit	Tregs+anti-CD20 Rituximab (N=12)				Tregs (N=13)				Control (N=11)			
	At Risk	Event	Censored	Survival	At Risk	Event	Censored	Survival	At Risk	Event	Censored	Survival
3-m	12	0	0	1.000	13	3	2	0.769	11	6	1	0.455
6-m	12	1	0	0.917	8	3	0	0.481	4	2	0	0.227
9-m	11	3	0	0.667	5	2	1	0.288	2	2	0	0.000
12-m	8	1	1	0.583	2	1	0	0.144				
15-m	6	1	0	0.486								
18-m	5	1	0	0.389								
24-m	4	1	3	0.292	1	0	1	0.144				

Abbreviations: Tregs: T regulatory cells, m: month, ITT: intention to treat, N: number of treated patients
Note(s): Survival – Duration of remission until first loss.
This table shows the results of a survival analysis with the time to first loss of remission status at the month 3 visit as the starting point. Visits 3m, 6m, 9m, 12m, 15m, 18m, 21m and 24m are included in the analysis.
If the time to first loss of remission status of a patient is not known for a scheduled visit, the patient will be censored from that visit onward.
Source: Table 14.2.1.6.5, Listing 16.2.6.9

Figure 5-7: Kaplan – Meier-Plot of Time to First Loss of Remission (ITT Set)



Abbreviations: Tregs: T regulatory cells, ITT: intention-to-treat
Note(s): Survival - Duration of remission until first loss.
This figure shows the results of a survival analysis with the time to first loss of remission status at the month 3 visit as the starting point.
Visits 3m, 6m, 9m, 12m, 15m, 18m, 21m and 24m are included in the analysis.
If the remission status of a subject is not known for a scheduled visit, the subject will be censored from that visit onward.
Source: Figure 14.2.1.6.7

5.1.2. Analysis of secondary endpoints

5.1.2.1. AUC of C-Peptide (MMTT, 0-240min, All Visits)

The geometric mean ratios of Tregs+anti-CD20 antibody rituximab/control and Tregs/control were obtained from the point estimates and confidence limits of repeated measurement mixed model ANCOVA (RMANCOVA) of the logarithmized AUC of C-peptide (MMTT, 0-240 min) values at all visits. The results showed that while the Tregs+anti-CD20 antibody rituximab group was statistically significantly superior to the control group at 12 (1.666, 90% CI 1.069-2.594), 18 (2.170, 90% CI 1.257-3.744) and 24 (1.955, 90% CI 1.058-3.613) months, the Tregs group was not superior to the control group at any timepoint, and the comparison of the two treatment groups showed that there was no statistically significant difference between the combined therapy and the monotherapy (Table 5-10, Figure 5-8). However, time plots of geometric mean AUC of C peptide levels (MMTT, 0-240 min) demonstrate that although AUC of C-peptide did decrease over time in all groups, it was consistently higher in both treatment groups than in the control group and consistently highest in the Tregs+anti-CD20 antibody rituximab group (Figure 5-9).

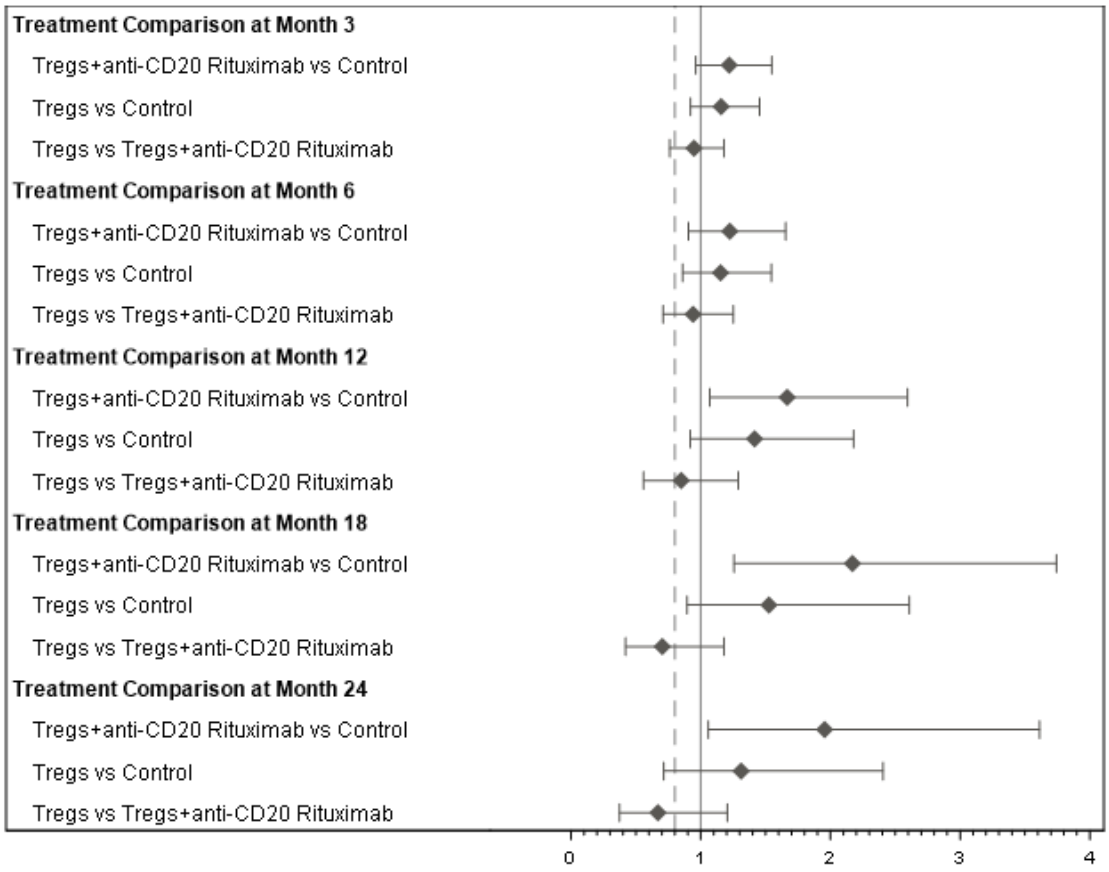
The AUC of C-peptide levels (MMTT, 0-240 min) obtained at all visits are listed by patient and visit in Listing 16.2.6.1 and tabulated in summary tables by treatment group in Table 14.2.2.1.1. RMANCOVA for logarithmized AUC of C-peptide (MMTT) values at all timepoints starting from month 3 is shown in Table 14.2.2.1.2. A Forest plot (RMANCOVA) of the geometric mean ratios of AUC of C-peptide (MMTT, 0-240 min) is shown in Figure 14.2.2.1.3. Mean AUC time plots of C-peptide levels (MMTT, 0-240 min) at all visits are presented graphically in the same graph overlaid for treatment group in Figures 14.2.1.1.5 and 14.2.1.1.6. Geometric mean AUC time plots of C-peptide levels (MMTT, 0-240 min) are provided in Figures 14.2.1.1.7 and 14.2.1.1.8)

Table 5-10: AUC of C-Peptide (MMTT, 0-240 min, All Visits) – RMANCOVA (ITT Set)

Visit	Comparison	Geometric mean		Treatment ratio	90% Confidence interval
		Test	Reference		
3 m	Tregs+anti-CD20 Rituximab vs Control	8.371	6.860	1.220	0.962 - 1.548
	Tregs vs Control	7.935	6.860	1.157	0.920 - 1.454
	Tregs vs Tregs+anti-CD20 Rituximab	7.935	8.371	0.948	0.761 - 1.180
6 m	Tregs+anti-CD20 Rituximab vs Control	7.648	6.252	1.223	0.904 - 1.655
	Tregs vs Control	7.203	6.252	1.152	0.860 - 1.544
	Tregs vs Tregs+anti-CD20 Rituximab	7.203	7.648	0.942	0.709 - 1.251
12 m	Tregs+anti-CD20 Rituximab vs Control	7.659	4.598	1.666	1.069 - 2.594*
	Tregs vs Control	6.504	4.598	1.415	0.919 - 2.179
	Tregs vs Tregs+anti-CD20 Rituximab	6.504	7.659	0.849	0.559 - 1.290
18 m	Tregs+anti-CD20 Rituximab vs Control	6.568	3.027	2.170	1.257 - 3.744*
	Tregs vs Control	4.621	3.027	1.527	0.894 - 2.607
	Tregs vs Tregs+anti-CD20 Rituximab	4.621	6.568	0.704	0.420 - 1.179
24 m	Tregs+anti-CD20 Rituximab vs Control	5.597	2.863	1.955	1.058 - 3.613*
	Tregs vs Control	3.753	2.863	1.311	0.714 - 2.406
	Tregs vs Tregs+anti-CD20 Rituximab	3.753	5.597	0.671	0.373 - 1.206

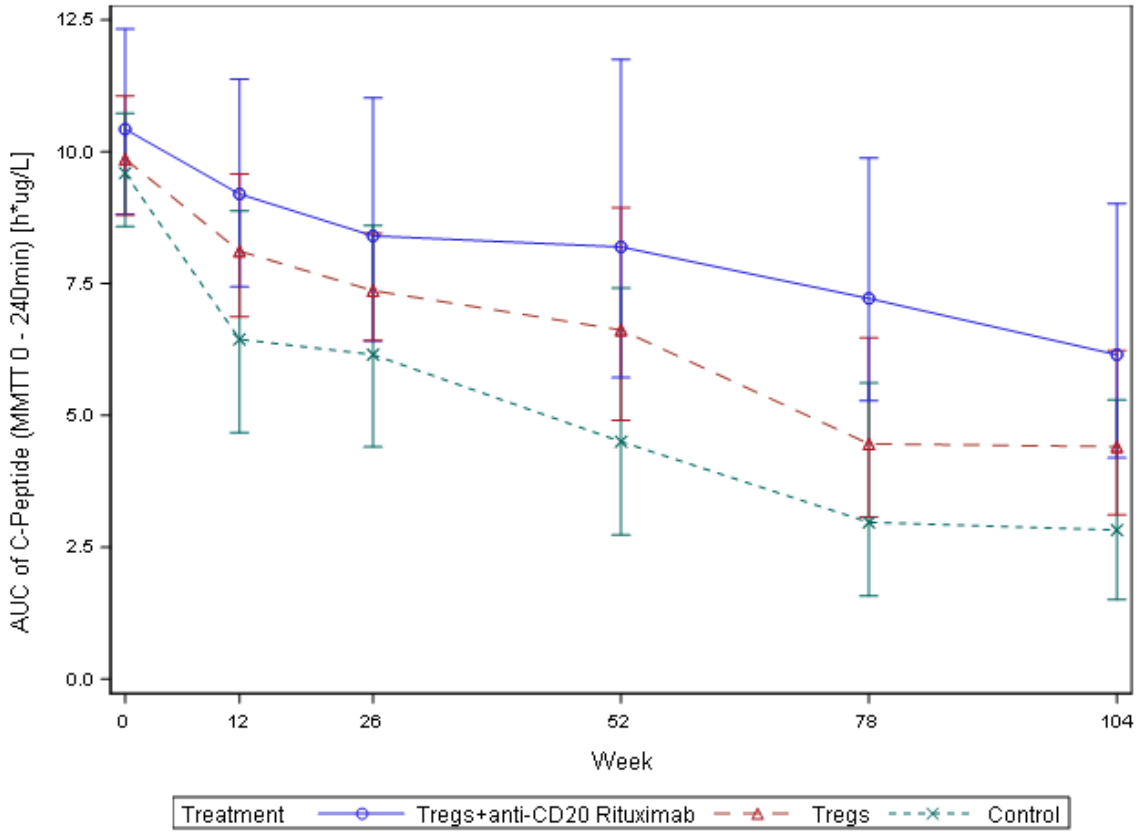
Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measure analysis of covariance, ITT: intention-to-treat, AUC: area under the curve, MMTT: mixed meal tolerance test, min: minutes, m: month
Note(s): * Indicates a significant difference (these values are boxed)
Results for a repeated measurement mixed model RMANCOVA for logarithmized AUC of C-peptide (0-240min) values, with log baseline value (prior to first treatment, Day 0) as continuous covariate, gender, age group (either <=12 years or > 12 years), treatment, visit and treatment*visit interaction as fixed categorical effects for the
within subject repeated measurements during follow up visits will be performed, i.e. visits 3m, 6m, 12m, 18m and 24m will be included in this analysis. Residuals were not normally distributed.
Source: Table 14.2.2.1.2, Listing 16.2.6.1

Figure 5-8: Forest Plot (RMANCOVA) of Geometric Mean Ratios of AUC of C-peptide (MMTT, 0-240 min) (ITT Set)



Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measure analysis of covariance, AUC: area under the curve, MMTT: mixed meal tolerance test, ITT: intention-to-treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented.
Source: Figure 14.2.2.1.3

Figure 5-9: Geometric Mean AUC Time Plots of C-peptide Levels (MMTT, 0-240 min) (ITT Set)



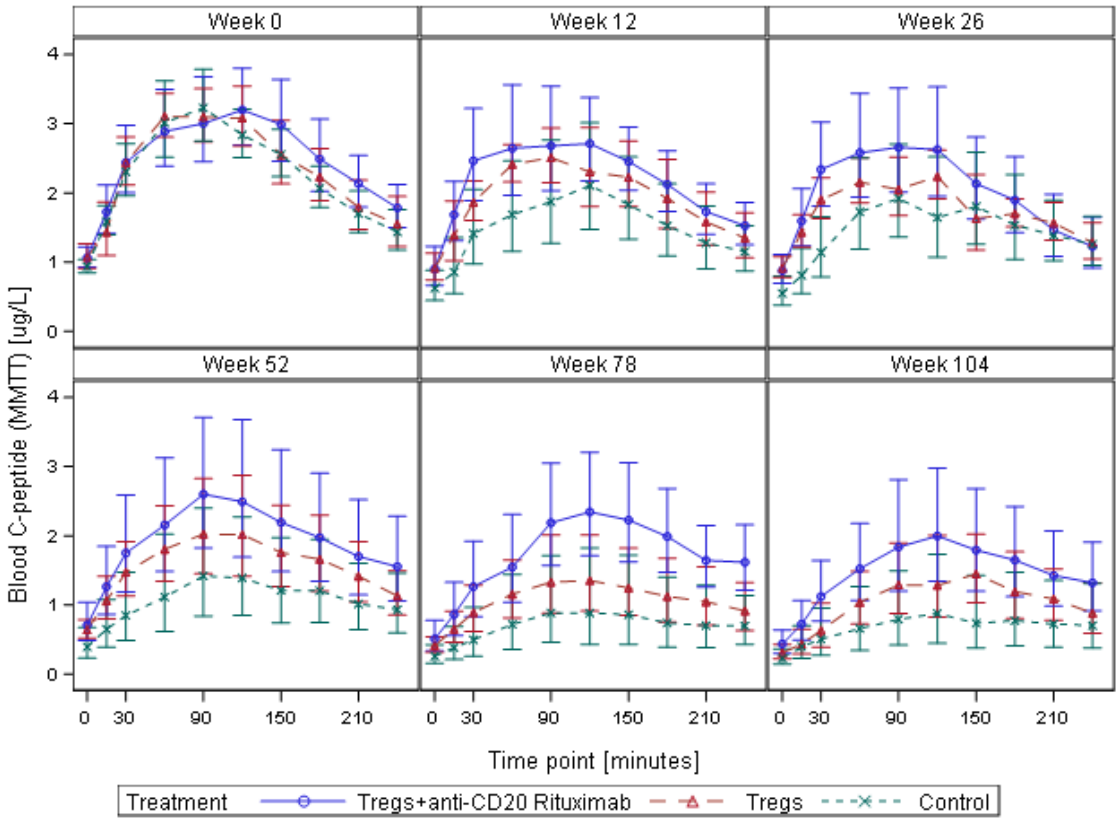
Abbreviations: AUC: area under the curve, MMTT: mixed meal tolerance test, min: minute, ITT: intention-to-treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented.
Source: Figure 14.2.1.1.7,

5.1.2.2. C-Peptide (MMTT, All Visits)

C-peptide levels were measured at 0, 15, 30, 60, 90, 120. 150, 180, 210 and 240 min after the start of MMTT. Geometric mean ratios for C-peptide (MMTT) at all visits were obtained using an RMANCOVA approach including repeated measurement effect for visits and measurement times. The results showed that of the 50 timepoints analyzed (10 timepoints at each of 5 visits: months 3,6,12,18, and 24), Tregs+anti-CD20 antibody rituximab was statistically significantly superior to the control treatment at 25 (50%), while Tregs was statistically significantly superior to the control at only 3 (6%). Most of the timepoints at which the combination therapy was superior to control (16, 64%) and all of those at which the monotherapy was superior to the control (3, 100%) were in the first 60 minutes of the MMTT. Although the non-inferiority analysis showed that there was no statistically significant difference between the groups (see Table 14.2.2.2.2 for individual geometric means with treatment ratios and 90% Cis and Figure 14.2.2.2.3 for Forest Plots of RMANCOVA of geometric mean ratios of C-peptide [MMTT, 0-240 min] at all visits), geometric mean concentration time plots of C-peptide levels in the MMTT (0-240 min) at all visits show that C-peptide levels were consistently higher throughout the study in both treatment groups, but they were consistently highest in the combination therapy group (Figure 5-10).

C-Peptide levels (MMTT, 0-240 min, all visits) are listed by patient and visit in Listing 16.2.6.2 and tabulated in a summary table by visit and treatment (Table 14.2.2.2.1). Mean (±SD) concentration time plots of C peptide levels (MMTT, 0-240 min) are shown by visit in Figures 14.2.1.3.5 and 14.2.1.3.6. Geometric mean concentration time plots are shown with 90% CIs in Figures 14.2.1.3.7 and 14.2.1.3.8.

Figure 5-10: Geometric Mean Concentration Time Plots of C-peptide Levels (MMTT, 0-240 min) (ITT Set)



Abbreviations: Tregs: T regulatory cells, MMTT: mixed meal tolerance test, ITT: intention-to-treat, min: minutes
Note(s): Geometric means and their 90 % confidence intervals are presented.
Source: Figure 14.2.1.3.7,

5.1.2.3. C-Peptide (Fasted, All Visits)

Geometric mean ratios for C-peptide (fasted) at all visits were obtained using an RMANCOVA approach including repeated measurement effect for visits and measurement times. The results showed that both Tregs+anti-CD20 antibody rituximab and Tregs alone was superior to the control at 3 months (1.729, 90% CI 1.229-2.433 and 1.811, 1.301-2.523, respectively), 6 months (2.638, 90% CI 1.81-3.84 and 2.727, 90% CI 1.897-3.920, respectively), 12 months (2.618, 90% CI 1.640-4.177 and 2.437, 90% CI 1.548-3.834, respectively), 15 months (2.478, 90% CI 1.539-3.989 and 1.773, 90% CI 1.111-2.830, respectively), and 18 months (3.901, 90% CI 2.508-6.066 and 2.457, 90% CI 1.597-3.781, respectively) (Table 5-11, Figure 5-11). The Tregs + anti-CD20 antibody rituximab group but not the Tregs group was statistically significantly better than the control group at 21 months

(2.919, 90% CI 1.70-5.00) and 24 months (2.574, 90% CI 1.489-4.449). The combination therapy appeared to have better results than the monotherapy at months 18 (0.630, 90% CI 0.416-0.955), 21 (0.511 90% CI 0.304-0.859), and 24 (0.528 90% CI 0.314-0.889), and this observation was supported by the finding of statistically significant inferiority of the monotherapy when compared to the combination therapy.

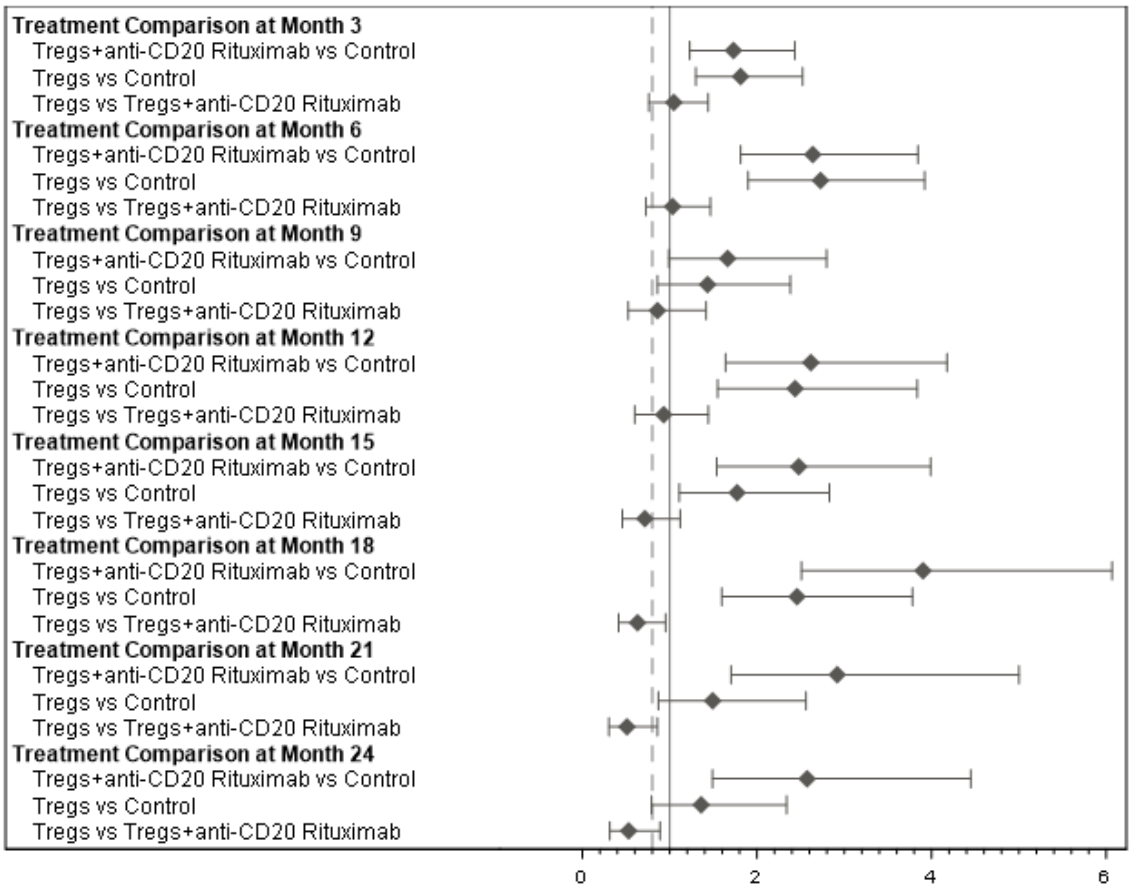
C-Peptide levels (fasted, all visits) are listed by patient and visit (Listing 16.2.6.3) and tabulated in a summary table by visit and treatment (Table 14.2.2.3.1). The RMANCOVA of C-peptide (fasted) values is shown in Table 14.2.2.3.2. A Forest plot of geometric mean ratios and their 90% CIs is shown in (Figure 14.2.2.3.3).

Table 5-11: C-Peptide (Fasted, All Visits) – RMANCOVA (ITT Set)

Visit	Comparison	Geometric mean		Treatment ratio	90% Confidence interval
		Test	Reference		
3 m	Tregs+anti-CD20 Rituximab vs Control	1.207	0.698	1.729	1.229 - 2.433*
	Tregs vs Control	1.264	0.698	1.811	1.301 - 2.523*
	Tregs vs Tregs+anti-CD20 Rituximab	1.264	1.207	1.047	0.762 - 1.440
6 m	Tregs+anti-CD20 Rituximab vs Control	1.188	0.450	2.638	1.811 - 3.843*
	Tregs vs Control	1.228	0.450	2.727	1.897 - 3.920*
	Tregs vs Tregs+anti-CD20 Rituximab	1.228	1.188	1.034	0.728 - 1.467
9-m	Tregs+anti-CD20 Rituximab vs Control	1.094	0.658	1.663	0.989 - 2.796
	Tregs vs Control	0.942	0.658	1.432	0.861 - 2.382
	Tregs vs Tregs+anti-CD20 Rituximab	0.942	1.094	0.861	0.524 - 1.416
12 m	Tregs+anti-CD20 Rituximab vs Control	0.983	0.375	2.618	1.640 - 4.177*
	Tregs vs Control	0.915	0.375	2.437	1.548 - 3.834*
	Tregs vs Tregs+anti-CD20 Rituximab	0.915	0.983	0.931	0.600 - 1.443
15-m	Tregs+anti-CD20 Rituximab vs Control	1.037	0.418	2.478	1.539 - 3.989*
	Tregs vs Control	0.742	0.418	1.773	1.111 - 2.830*
	Tregs vs Tregs+anti-CD20 Rituximab	0.742	1.037	0.715	0.457 - 1.120
18 m	Tregs+anti-CD20 Rituximab vs Control	0.963	0.247	3.901	2.508 - 6.066*
	Tregs vs Control	0.607	0.247	2.457	1.597 - 3.781*
	Tregs vs Tregs+anti-CD20 Rituximab	0.607	0.963	0.630	0.416 - 0.955*
21-m	Tregs+anti-CD20 Rituximab vs Control	0.925	0.317	2.919	1.703 - 5.002*
	Tregs vs Control	0.473	0.317	1.492	0.870 - 2.561
	Tregs vs Tregs+anti-CD20 Rituximab	0.473	0.925	0.511	0.304 - 0.859*
24 m	Tregs+anti-CD20 Rituximab vs Control	0.753	0.292	2.574	1.489 - 4.449*
	Tregs vs Control	0.398	0.292	1.360	0.791 - 2.339
	Tregs vs Tregs+anti-CD20 Rituximab	0.398	0.753	0.528	0.314 - 0.889*

Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measures analysis of covariance, ITT: intention-to-treat
Notes: *indicates a significant difference (these values are boxed).
Results for a repeated measurement mixed model RMANCOVA for logarithmized C-peptide (Fasted) values, with log baseline value (prior to first treatment, Day 0) as continuous covariate, gender, age group (either <=12 years or > 12 years), treatment, visit and treatment*visit interaction as fixed categorical effects for the within subject repeated measurements during follow-up visits will be performed, i.e. visits 3m, 6m, 9m, 12m, 15m, 18m, 21m and 24m will be included in this analysis. Residuals were not normally distributed.
Source: Table 14.2.2.3.2, Listing 16.2.6.3

Figure 5-11: Forest Plot (RMANCOVA) of Geometric Mean Ratios of C-peptide (Fasted) (ITT Set)



Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measures analysis of covariance, ITT: intention-to-treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented
Source: Figure 14.2.2.3.3

5.1.2.4. C-Peptide (Glucagon Test, All Visits)

Geometric mean ratios for C-peptide (glucagon test) at all visits and AUC of C-peptide (glucagon test) at all visits were obtained using a statistical approach (RMANCOVA) similar to that described in Section 5.1.2.1.

AUC of C peptide (glucagon test) was statistically significantly superior in the Tregs+anti-CD20 antibody rituximab group in comparison to the control group at 6 months (1.722, 90% CI 1.119-2.649), 12 months (1.803, 90% CI 1.121-2.900), and 18 months (2.722, 90% CI 1.649-4.494) and in the Tregs group than the control group at only the month 18 visit (1.653, 90% CI 1.013-2.698). At month 24, although the difference between either treatment group and the control was not statistically significant, the outcome for the monotherapy was shown to be statistically significantly inferior to the combined therapy (0.408, 90% CI 0.238-0.701; Table 5-12, Figure 5-12). Indeed, the Forest plot (RMANCOVA) of geometric mean ratios of AUC of C peptide (glucagon test) shows that values were consistently higher, and sometimes much higher (e.g., at 18 months), in the combined therapy group than in the monotherapy

group, and there is a clear tendency for the combination therapy group to become increasingly better than the monotherapy (Figure 5-12).

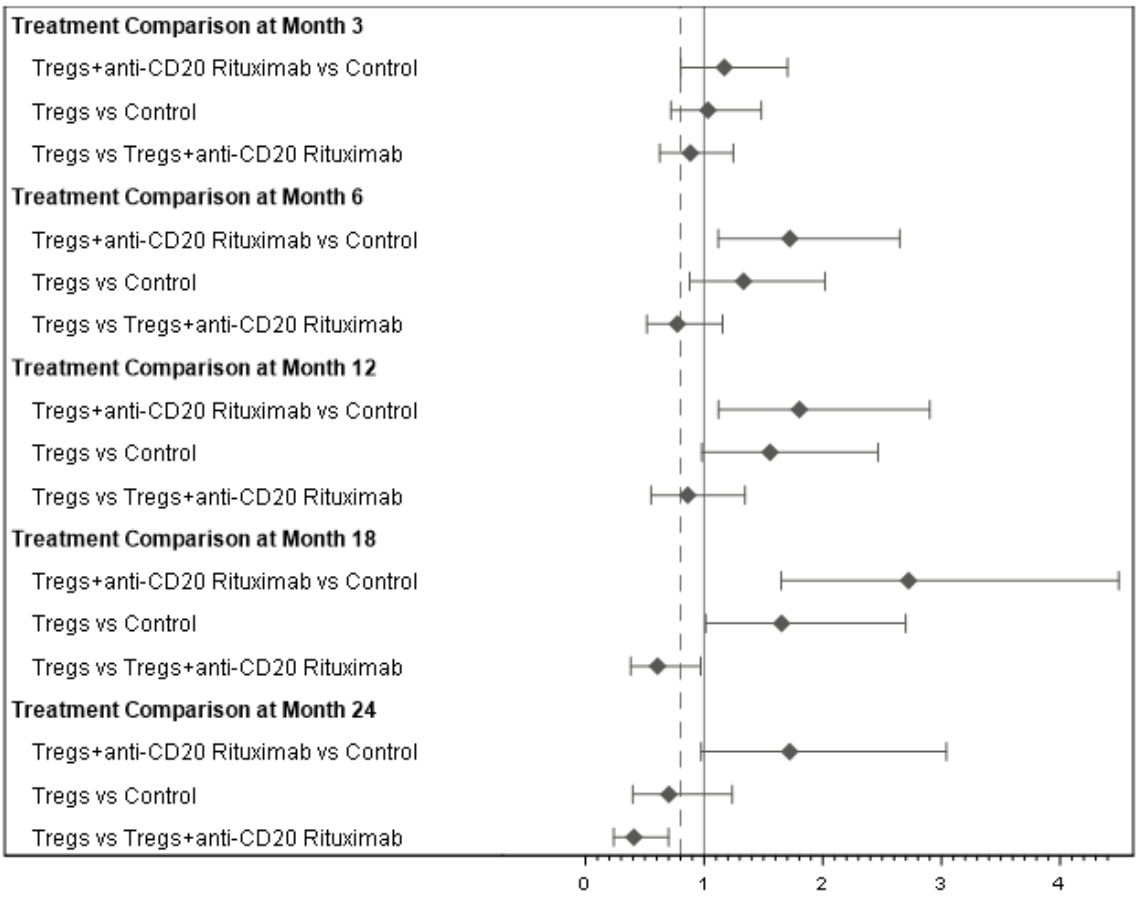
AUC of C-peptide (Glucagon test, 0-6 min, month 3 to month 24 visits) is listed by patient and visit (Listing 16.2.6.5) and tabulated in a summary table by visit and treatment (Table 14.2.2.4.1). The RMANCOVA of AUC of C peptide (glucagon test, 0-6 min) at all visits is shown in Table 14.2.2.4.2. The Forest Plot for this RMANCOVA is shown in Figure 14.2.2.4.3. Mean AUC time plots of C peptide levels (glucagon test, 0-6 min) are shown in Figures 14.2.1.2.5 and 14.2.1.2.6. Geometric mean AUC time plots of C peptide (glucagon, 0-6 min) are shown in Figures 14.2.1.2.7 and 14.2.1.2.8.

Table 5-12: AUC of C-Peptide (Glucagon Test, 0-6min, All Visits) – RMANCOVA (ITT Set)

Visit	Comparison	Geometric mean		Treatment ratio	90% Confidence interval
		Test	Reference		
3 m	Tregs+anti-CD20 Rituximab vs Control	0.146	0.125	1.169	0.803 - 1.703
	Tregs vs Control	0.129	0.125	1.032	0.719 - 1.482
	Tregs vs Tregs+anti-CD20 Rituximab	0.129	0.146	0.883	0.625 - 1.246
6 m	Tregs+anti-CD20 Rituximab vs Control	0.147	0.086	1.722	1.119 - 2.649*
	Tregs vs Control	0.114	0.086	1.331	0.878 - 2.018
	Tregs vs Tregs+anti-CD20 Rituximab	0.114	0.147	0.773	0.517 - 1.155
12 m	Tregs+anti-CD20 Rituximab vs Control	0.114	0.063	1.803	1.121 - 2.900*
	Tregs vs Control	0.098	0.063	1.555	0.980 - 2.465
	Tregs vs Tregs+anti-CD20 Rituximab	0.098	0.114	0.862	0.553 - 1.345
18 m	Tregs+anti-CD20 Rituximab vs Control	0.095	0.035	2.722	1.649 - 4.494*
	Tregs vs Control	0.058	0.035	1.653	1.013 - 2.698*
	Tregs vs Tregs+anti-CD20 Rituximab	0.058	0.095	0.607	0.380 - 0.970
24 m	Tregs+anti-CD20 Rituximab vs Control	0.084	0.049	1.721	0.974 - 3.042
	Tregs vs Control	0.034	0.049	0.702	0.399 - 1.236
	Tregs vs Tregs+anti-CD20 Rituximab	0.034	0.084	0.408	0.238 - 0.701*

Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measures analysis of covariance, ITT: intention-to-treat, AUC: area under the curve
Note(s): *indicates a significant difference (these values are boxed).
Results for a repeated measurement mixed model RMANCOVA for logarithmized AUC of C-peptide (Glucagon Test, 0-6min) values, with log baseline value (prior to first treatment, Day 0) as continuous covariate, gender, age group (either <=12 years or > 12 years), treatment, visit and treatment*visit interaction as fixed categorical effects for the within subject repeated measurements during follow up visits will be performed, i.e. visits 3m, 6m, 12m, 18m and 24m will be included in this analysis.
Residuals were normally distributed.
Source: Table 14.2.2.4.2, Listing 16.2.6.5

Figure 5-12: Forest Plot (RMANCOVA) of Geometric Mean Ratios of AUC of C peptide (Glucagon test, 0-6 min) (ITT Set)



Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measures analysis of covariance, AUC: area under the curve, ITT: intention to treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented.
Source: Figure 14.2.2.4.3

C peptide levels (glucagon test, all visits) were statistically significantly superior to the control group in the Tregs+anti-CD20 antibody rituximab group at the 0-minute timepoint at 6 months (1.532, 90% CI 1.022-2.295), 18 months (2.591, 90% CI 1.615-4.154), and 24 months (1.717, 90% CI 1.019-2.896) and at the 6-minute timepoint at 12 months (1.738, 90% CI 1.012-2.986) and 18 months (2.431, 90% CI 1.371-4.309). In the Tregs group, levels were statistically significantly superior to the control group at only one timepoint: 0 minutes at the 18-month visit (1.630, 90% CI 1.026-2.590). Furthermore, the levels in the Tregs group were inferior to those in the Tregs Tregs+anti-CD20 antibody rituximab group at 0 minutes in the 18- and 24-month visits and at 6 minutes at the 24-month visit (Table 5-13)..

Descriptive statistics for C-Peptide (glucagon test, 0 min and 6 min) at the month 3 to month 24 visits are summarized in Table 14.2.2.10.1. The results of RMANCOVA showing treatment comparisons of geometric mean ratios are shown in Table 14.2.2.10.2. The Forest plot (RMANCOVA) of geometric mean ratios of C peptide (glucagon test) at the month 3 to month 24 visits is shown in Figure 14.2.2.10.3. Mean concentration time plots of C peptide

(glucagon test) are shown in Figures 14.2.1.7.5 and 14.2.1.7.6. Geometric mean concentration time plots of C peptide levels (glucagon test) are shown in Figures 14.2.1.7.7 and 14.2.1.7.8.

Table 5-13: C-Peptide (Glucagon Test, All Visits) – RMANCOVA (ITT Set)

Min	Visit	Comparison	Geometric mean		Treatment ratio	90% Confidence interval
			Test	Reference		
0	3 m	Tregs+anti-CD20 Rituximab vs Control	0.856	0.817	1.048	0.762 - 1.440
		Tregs vs Control	0.826	0.817	1.010	0.743 - 1.373
		Tregs vs Tregs+anti-CD20 Rituximab	0.826	0.856	0.964	0.726 - 1.281
	6 m	Tregs+anti-CD20 Rituximab vs Control	0.883	0.576	1.532	1.022 - 2.295*
		Tregs vs Control	0.810	0.576	1.405	0.949 - 2.080
		Tregs vs Tregs+anti-CD20 Rituximab	0.810	0.883	0.917	0.631 - 1.333
	12 m	Tregs+anti-CD20 Rituximab vs Control	0.709	0.473	1.499	0.963 - 2.335
		Tregs vs Control	0.683	0.473	1.444	0.939 - 2.221
		Tregs vs Tregs+anti-CD20 Rituximab	0.683	0.709	0.963	0.639 - 1.452
	18 m	Tregs+anti-CD20 Rituximab vs Control	0.709	0.274	2.591	1.615 - 4.154*
		Tregs vs Control	0.446	0.274	1.630	1.026 - 2.590*
		Tregs vs Tregs+anti-CD20 Rituximab	0.446	0.709	0.629	0.406 - 0.975*
	24 m	Tregs+anti-CD20 Rituximab vs Control	0.579	0.337	1.717	1.019 - 2.896*
		Tregs vs Control	0.271	0.337	0.805	0.476 - 1.360
		Tregs vs Tregs+anti-CD20 Rituximab	0.271	0.579	0.469	0.285 - 0.770*
6	3 m	Tregs+anti-CD20 Rituximab vs Control	1.816	1.701	1.068	0.653 - 1.747
		Tregs vs Control	1.637	1.701	0.962	0.598 - 1.549
		Tregs vs Tregs+anti-CD20 Rituximab	1.637	1.816	0.901	0.579 - 1.404
	6 m	Tregs+anti-CD20 Rituximab vs Control	1.857	1.149	1.615	0.966 - 2.703
		Tregs vs Control	1.321	1.149	1.149	0.699 - 1.891
		Tregs vs Tregs+anti-CD20 Rituximab	1.321	1.857	0.711	0.444 - 1.139
	12 m	Tregs+anti-CD20 Rituximab vs Control	1.388	0.799	1.738	1.012 - 2.986*
		Tregs vs Control	1.192	0.799	1.492	0.883 - 2.522
		Tregs vs Tregs+anti-CD20 Rituximab	1.192	1.388	0.858	0.523 - 1.409
	18 m	Tregs+anti-CD20 Rituximab vs Control	1.058	0.435	2.431	1.371 - 4.309*
		Tregs vs Control	0.654	0.435	1.503	0.858 - 2.630
		Tregs vs Tregs+anti-CD20 Rituximab	0.654	1.058	0.618	0.365 - 1.046
	24 m	Tregs+anti-CD20 Rituximab vs Control	0.983	0.632	1.555	0.810 - 2.986
		Tregs vs Control	0.386	0.632	0.611	0.319 - 1.169
		Tregs vs Tregs+anti-CD20 Rituximab	0.386	0.983	0.393	0.213 - 0.723*

Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measure analysis of covariance, ITT: intention-to-treat, min: minutes.
Note(s): *indicates a significant difference (these values are boxed)
Results for a repeated measurement mixed model RMANCOVA for logarithmized C-peptide (Glucagon Test) values by time point, with log baseline value (prior to first treatment, Day 0) as continuous covariate, gender, age group (either <=12 years or > 12 years), treatment, visit and treatment*visit interaction as fixed categorical effects for the within subject repeated measurements during follow up visits will be performed, i.e. visits 3m, 6m, 12m, 18m and 24m will be included in this analysis. Residuals were normally distributed
Source: Table 14.2.2.10.2, Listing 16.2.6.11

5.1.2.5. HbA1c (All Visits)

Geometric mean ratios for HbA1c at all visits were obtained using a statistical approach (RMANCOVA) similar to that described in Section 5.1.2.1

RMANCOVA of HbA1c levels at the month 3 to 24 visits showed that Tregs+anti-CD20 antibody rituximab was statistically significantly superior to the control group (unity completely below 1) at months 3, 6, 9, 12, 21, and 24, while Tregs was statistically

significantly superior to the control only at the month 3 visit. Importantly, non-inferiority analysis showed that despite the many statistically significant comparisons of the combination therapy vs. control, the monotherapy was statistically not inferior to the combination therapy at every timepoint (all 95% CIs completely below the non-inferiority margin) except at month 9 where non-inferiority of the monotherapy vs. the combined therapy could not be shown (Table 5-14, Figure 5-13). The mean concentration time plot of HbA1c provides further support for this finding with its widely overlapping standard deviations.(Figure 5-14).

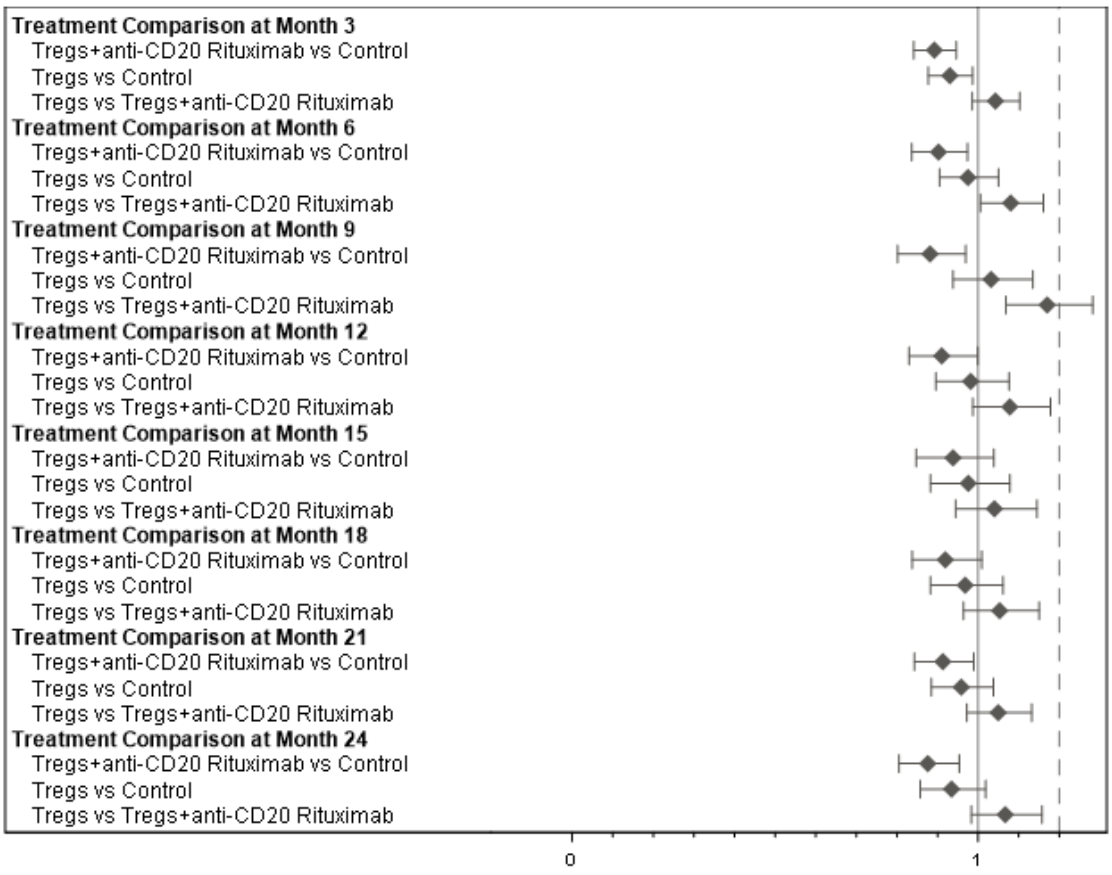
HbA1c values (month 3 to 24 visits) are listed by patient and visit (Listing 16.2.6.6) and tabulated in a summary table by visit and treatment (Table 14.2.2.5.1). RMANCOVA of geometric mean ratios and their 90% CIs is shown in Table 14.2.2.5.2. A Forest plot shows the treatment ratios with 90% CIs (Figure 14.2.2.5.3). Mean concentration time plots with SD intervals and a geometric mean plot with 90% CIs are also provided (Figures 14.2.2.5.4 and 14.2.2.5.5, respectively).

Table 5-14: HbA1c (All Visits) – RMANCOVA (ITT Set)

Visit	Comparison	Geometric mean		Treatment ratio	90% Confidence interval
		Test	Reference		
3 m	Tregs+anti-CD20 Rituximab vs Control	5.923	6.637	0.892	0.842 - 0.946*
	Tregs vs Control	6.174	6.637	0.930	0.877 - 0.987*
	Tregs vs Tregs+anti-CD20 Rituximab	6.174	5.923	1.042	0.985 - 1.103*
6 m	Tregs+anti-CD20 Rituximab vs Control	5.956	6.600	0.902	0.836 - 0.974*
	Tregs vs Control	6.436	6.600	0.975	0.905 - 1.051
	Tregs vs Tregs+anti-CD20 Rituximab	6.436	5.956	1.081	1.006 - 1.161*
9 m	Tregs+anti-CD20 Rituximab vs Control	5.823	6.605	0.882	0.802 - 0.970*
	Tregs vs Control	6.816	6.605	1.032	0.938 - 1.135
	Tregs vs Tregs+anti-CD20 Rituximab	6.816	5.823	1.170	1.068 - 1.282
12 m	Tregs+anti-CD20 Rituximab vs Control	6.129	6.731	0.911	0.830 - 0.999*
	Tregs vs Control	6.609	6.731	0.982	0.896 - 1.076
	Tregs vs Tregs+anti-CD20 Rituximab	6.609	6.129	1.078	0.987 - 1.178*
15 m	Tregs+anti-CD20 Rituximab vs Control	6.341	6.759	0.938	0.847 - 1.039
	Tregs vs Control	6.595	6.759	0.976	0.883 - 1.079
	Tregs vs Tregs+anti-CD20 Rituximab	6.595	6.341	1.040	0.945 - 1.145*
18 m	Tregs+anti-CD20 Rituximab vs Control	6.300	6.853	0.919	0.837 - 1.009
	Tregs vs Control	6.634	6.853	0.968	0.883 - 1.062
	Tregs vs Tregs+anti-CD20 Rituximab	6.634	6.300	1.053	0.964 - 1.150*
21 m	Tregs+anti-CD20 Rituximab vs Control	6.308	6.907	0.913	0.843 - 0.989*
	Tregs vs Control	6.619	6.907	0.958	0.885 - 1.038
	Tregs vs Tregs+anti-CD20 Rituximab	6.619	6.308	1.049	0.972 - 1.132*
24 m	Tregs+anti-CD20 Rituximab vs Control	6.137	7.004	0.876	0.805 - 0.954*
	Tregs vs Control	6.546	7.004	0.935	0.857 - 1.019
	Tregs vs Tregs+anti-CD20 Rituximab	6.546	6.137	1.067	0.983 - 1.157*

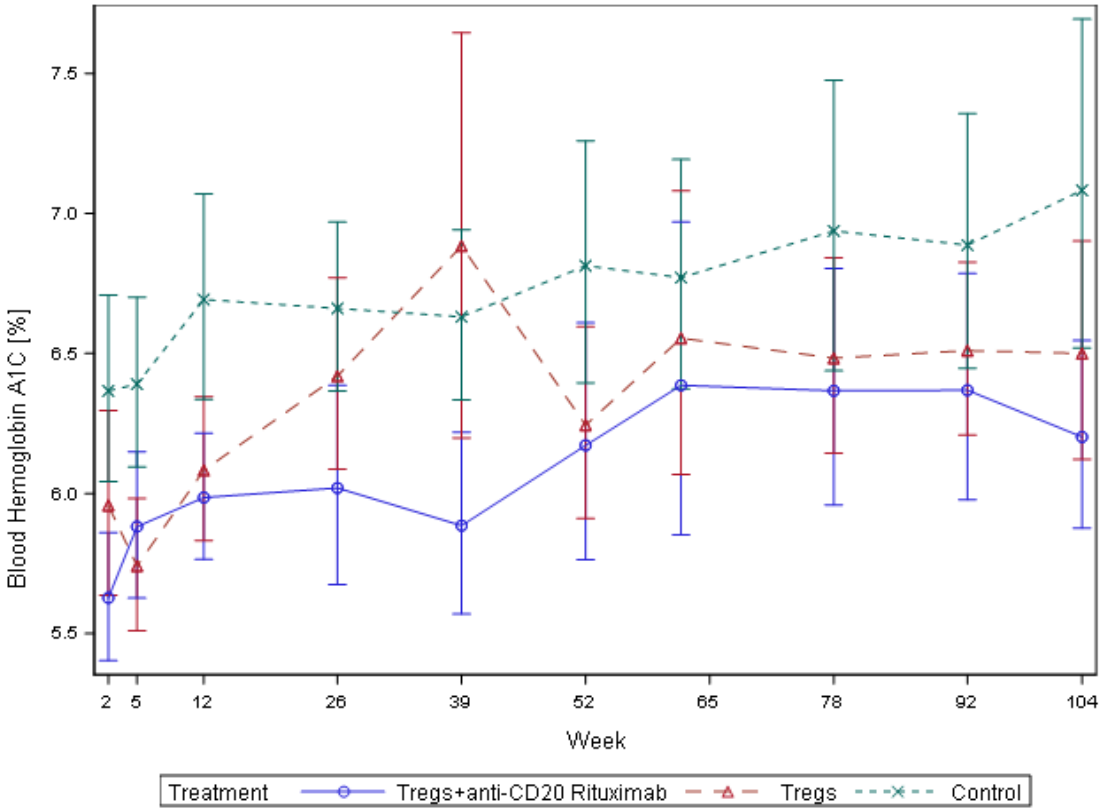
Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measures analysis of covariance, ITT: intention-to-treat
Note(s): * indicates a significant difference (these values are boxed).
Results for a repeated measurement mixed model RMANCOVA for logarithmized HBA1C values, with log baseline value (last value prior to first treatment, Day 0) as continuous covariate, gender, age group (either <=12 years or > 12 years), treatment, visit and treatment*visit interaction as fixed categorical effects for the within subject repeated measurements during follow up visits will be performed, i.e. visits 3m, 6m, 9m, 12m, 15m, 18m, 21m and 24m will be included in this analysis. Residuals were not normally distributed.
Source: Table 14.2.2.5.2, Listing 16.2.6.6

Figure 5-13: Forest Plot (RMANCOVA) of Geometric Mean Ratios of HbA1c (ITT Set)



Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measures analysis of covariance, ITT: intention-to-treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented
Source: Figure 14.2.2.5.3

Figure 5-14: Mean Concentration Time Plots of HbA1c (ITT Set)



Abbreviations: Tregs: T regulatory cells, ITT: intention-to-treat
Note(s): Means ± SD are presented.
Source: Figure 14.2.2.5.5

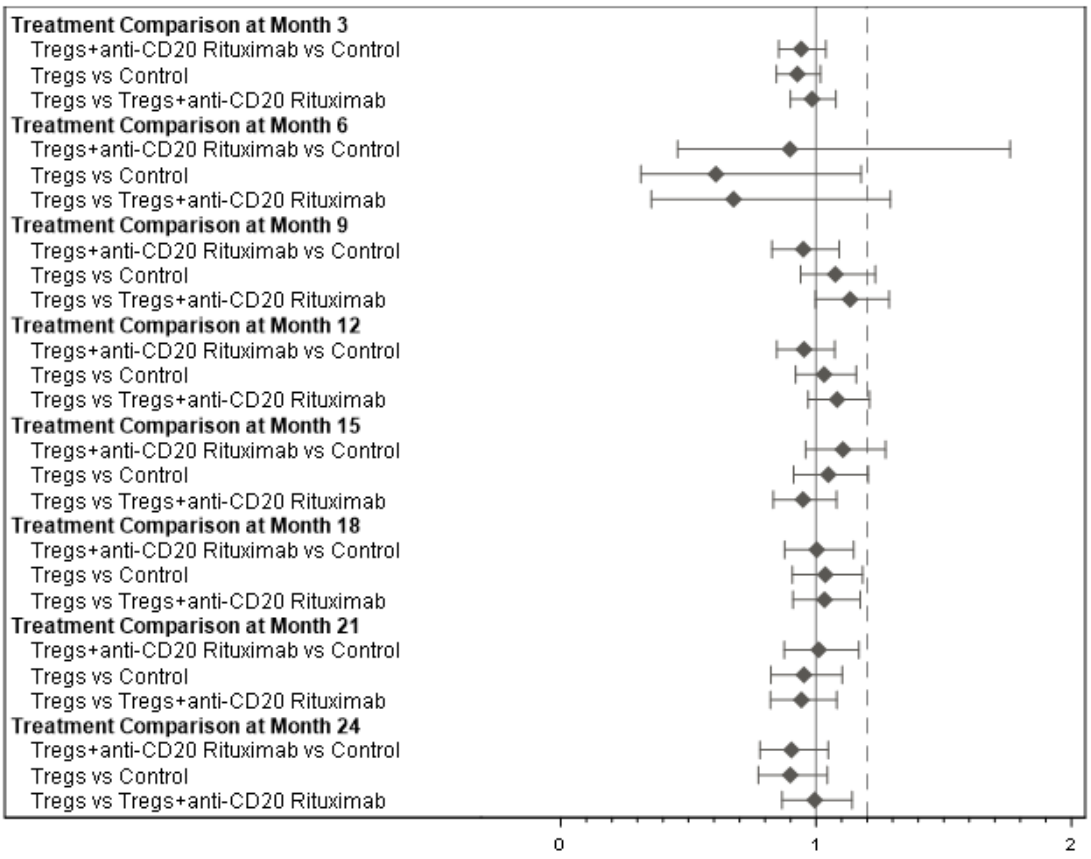
5.1.2.6. Glucose (All Visits)

Geometric mean ratios for glucose at all visits were obtained using a statistical approach (RMANCOVA) similar to that described in Section 5.1.2.1

Glucose levels were not statistically significantly different between any of the groups at any timepoint. This includes most of the comparisons between the two treatment groups, which showed non-inferiority of the monotherapy vs. the combination therapy at months 3, 15, 18 and 24.

Glucose levels (month 3 to month 24 visits) are listed by patient and visit (Listing 16.2.6.7) and tabulated in a summary table by visit and treatment (Table 14.2.2.6.1). RMANCOVA was performed on an exploratory basis based on logarithmized values (Table 14.2.2.6.2). A Forest plot (Figure 14.2.2.6.3) shows the treatment ratio estimates and 90% CIs for this analysis. A mean concentration time plot with SD intervals and a geometric mean plot with 90% CIs are also provided for glucose levels (Figure 14.2.2.6.4 and 14.2.2.6.5).

Figure 5-15: Forest Plot (RMANCOVA) of Geometric Mean Ratios of Glucose (ITT Set)



Abbreviations: Tregs: T regulatory cells, ITT: intention-to-treat
Note(s): Geometric mean ratios and 90 % confidence intervals are presented.
Source: Figure 14.2.2.6.3

5.1.2.7. Daily Insulin Dose per kg Body Weight (DDI, All Visits)

The point estimates for the treatment differences Tregs+anti-CD20 antibody rituximab vs. control and Tregs vs. control (ANCOVA) showed that although mean DDI increased during the study in all groups, the Tregs + anti-CD20 antibody rituximab group was statistically significantly superior to the control group (90% CI completely below 0) at month 6 (-0.144, 90% CI -0.276 - -0.012), month 12 (-0.233, 90% CI -0.386 - -0.079), month 15 (-0.243, 90% CI -0.385 - -0.100), month 18 (-0.205, 90% CI -0.376 - -0.035), month 21 (-0.287, 90% CI -0.460 - -0.114), and month 24 (-0.173, 90% CI -0.345 - -0.002), while the Tregs group performed statistically significantly better than the control group only at month 15 (-0.163, 90% CI -0.309 - -0.016) and month 21 (-0.287, 90% CI -0.441 - -0.082). Although these results suggest that the combination therapy might be superior in reducing DDI, comparison of the two treatments revealed no statistically significant difference at any timepoint (Table 5-15, Figure 5-16). The geometric mean concentration time plot of DDI reflects these results: although DDI increased over time in all three groups, DDI was consistently lowest in the combination therapy group, although at most timepoints its standard deviations overlapped those of the monotherapy group (Figure 5-17).

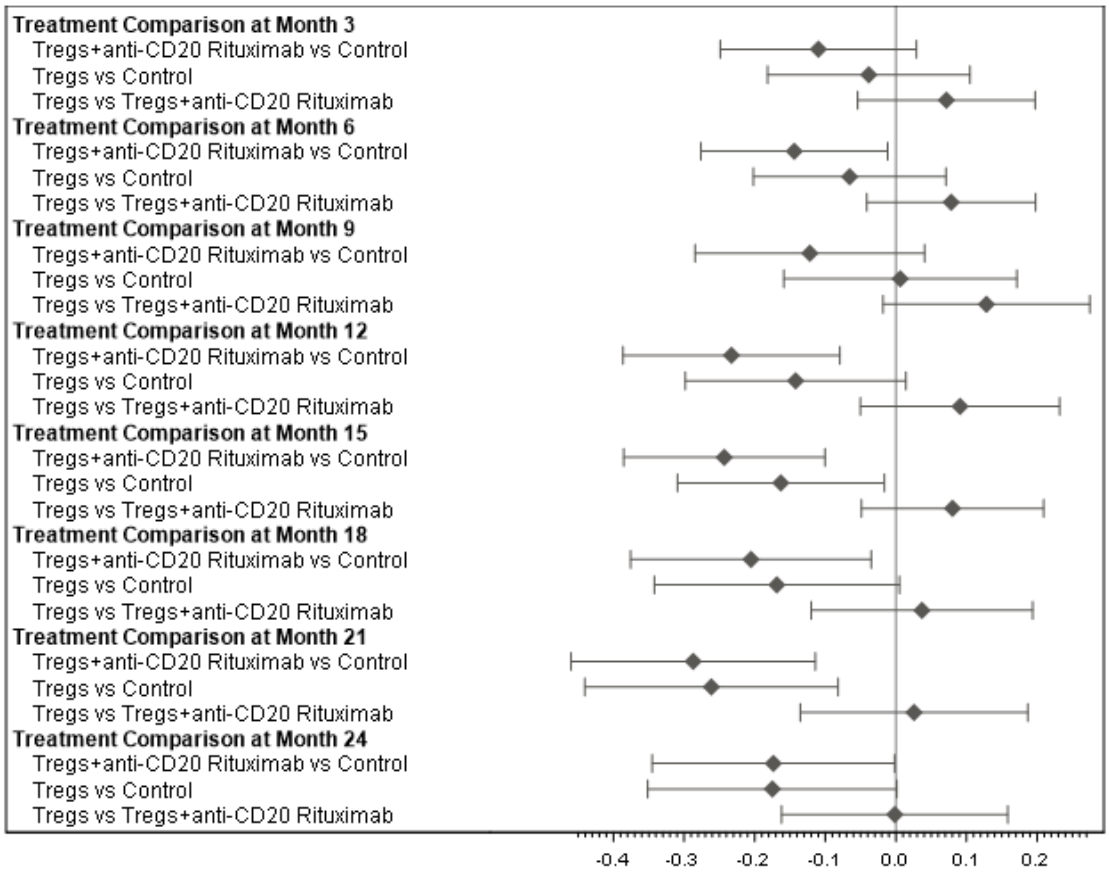
DDI values (month 3 to month 24 visits) are listed by patient and visit (Listing 16.2.6.8) and are tabulated in a summary table by visit and treatment (Table 14.2.2.7.1). The proportion of patients with DDI < 0.5UI/kg b.w. is displayed in a frequency table by visit and treatment (Table 14.2.2.7.3). Geometric mean concentration time plots of DDI are shown in Figure 14.2.1.5.7 and 14.2.1.5.8. An exploratory RMANCOVA analysis was performed to evaluate treatment differences in DDI at all visits (Table 14.2.2.7.2). A Forest plot (Figure 14.2.2.7.4) shows treatment difference estimates and their 90% CIs.

Table 5-15: Daily Insulin Dose per kg Body Weight (All Visits) – RMANCOVA (ITT Set)

Visit	Comparison	Mean		Treatment Difference	90% Confidence interval
		Test	Reference		
3 m	Tregs+anti-CD20 Rituximab vs Control	0.197	0.307	-0.110	-0.249 - 0.029
	Tregs vs Control	0.269	0.307	-0.038	-0.182 - 0.105
	Tregs vs Tregs+anti-CD20 Rituximab	0.269	0.197	0.072	-0.054 - 0.197
6 m	Tregs+anti-CD20 Rituximab vs Control	0.217	0.361	-0.144	-0.276 - -0.012*
	Tregs vs Control	0.296	0.361	-0.066	-0.202 - 0.071
	Tregs vs Tregs+anti-CD20 Rituximab	0.296	0.217	0.078	-0.041 - 0.198
9 m	Tregs+anti-CD20 Rituximab vs Control	0.283	0.405	-0.122	-0.284 - 0.041
	Tregs vs Control	0.412	0.405	0.006	-0.159 - 0.171
	Tregs vs Tregs+anti-CD20 Rituximab	0.412	0.283	0.128	-0.018 - 0.275
12 m	Tregs+anti-CD20 Rituximab vs Control	0.281	0.514	-0.233	-0.386 - -0.079*
	Tregs vs Control	0.372	0.514	-0.142	-0.298 - 0.014
	Tregs vs Tregs+anti-CD20 Rituximab	0.372	0.281	0.091	-0.050 - 0.232
15 m	Tregs+anti-CD20 Rituximab vs Control	0.339	0.582	-0.243	-0.385 - -0.100*
	Tregs vs Control	0.419	0.582	-0.163	-0.309 - -0.016*
	Tregs vs Tregs+anti-CD20 Rituximab	0.419	0.339	0.080	-0.049 - 0.209
18 m	Tregs+anti-CD20 Rituximab vs Control	0.482	0.688	-0.205	-0.376 - -0.035*
	Tregs vs Control	0.519	0.688	-0.168	-0.342 - 0.005
	Tregs vs Tregs+anti-CD20 Rituximab	0.519	0.482	0.037	-0.120 - 0.194
21 m	Tregs+anti-CD20 Rituximab vs Control	0.493	0.780	-0.287	-0.460 - -0.114*
	Tregs vs Control	0.519	0.780	-0.261	-0.441 - -0.082*
	Tregs vs Tregs+anti-CD20 Rituximab	0.519	0.493	0.026	-0.135 - 0.187
24 m	Tregs+anti-CD20 Rituximab vs Control	0.545	0.718	-0.173	-0.345 - -0.002*
	Tregs vs Control	0.543	0.718	-0.175	-0.352 - 0.001
	Tregs vs Tregs+anti-CD20 Rituximab	0.543	0.545	-0.002	-0.162 - 0.159

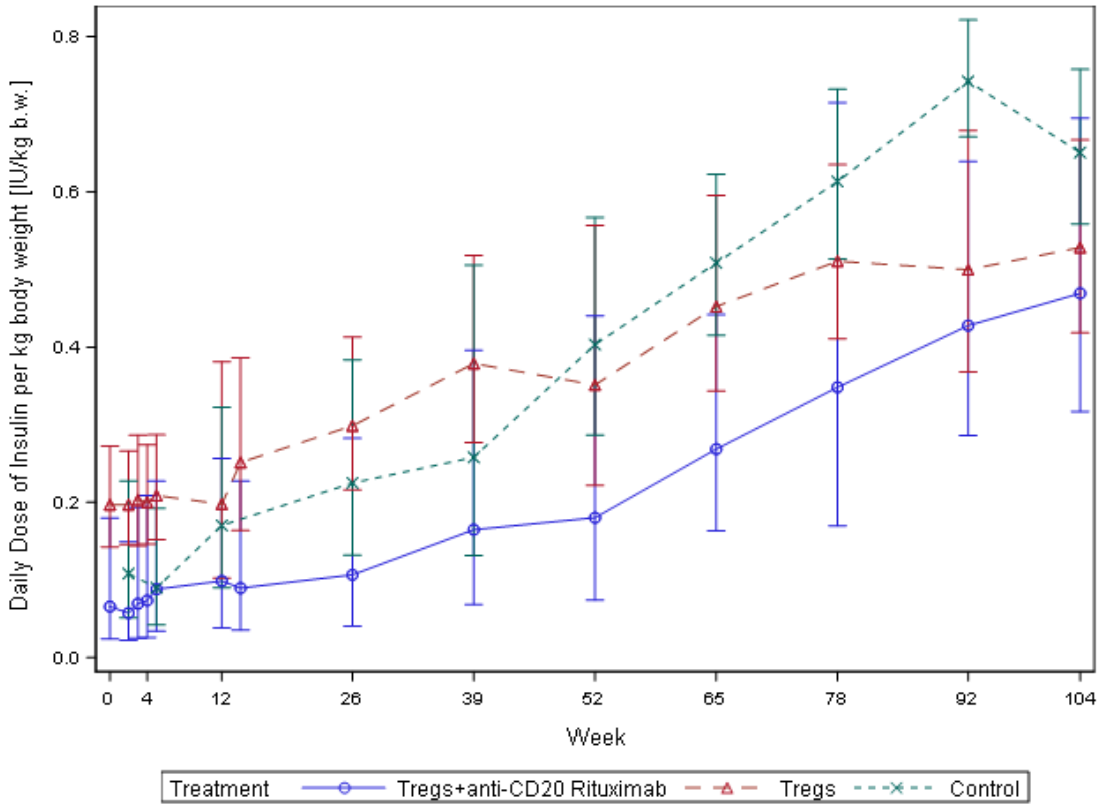
Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measures analysis of covariance, ITT: intention-to-treat, kg: kilogram
Note(s): * indicates a significant difference (these values are boxed).
Results for a repeated measurement mixed model RMANCOVA for daily insulin by body weight values, with baseline value (last value prior to first treatment, Day 0) as continuous covariate, gender, age group (either <=12 years or > 12 years), treatment, visit and treatment*visit interaction as fixed categorical effects for the within subject repeated measurements during follow-up visits will be performed, i.e. visits 3m, 6m, 9m, 12m, 15m, 18m, 21m and 24m will be included in this analysis. Visit Month 3 prim is not included in the analysis, because the control group does not have data for this visit. Residuals were not normally distributed.
Source: Table 14.2.2.7.2, Listing 16.2.6.8

Figure 5-16: Forest Plot (RMANCOVA) of Arithmetic Mean Differences of Daily Insulin Dose per kg body weight (ITT set)



Abbreviations: Tregs: T regulatory cells, RMANCOVA: repeated measures analysis of covariance, ITT: intention-to-treat, kg: kilogram
Note(s): Differences of the treatment means and 90 % confidence intervals are presented
Source: Figure 14.2.2.7.4

Figure 5-17: Geometric Mean Concentration Time Plots of Daily Insulin Dose per kg Body Weight (ITT Set)



Abbreviations: Tregs: T regulatory cells, ITT: intention-to-treat, kg b.w.: kilogram body weight
Note(s): Geometric means and their 90 % confidence intervals are presented.
Zero values were set to 0.005 (= half of the lowest non-zero value present in the data) for the calculation of the geometric means and their confidence intervals.
Source: Figure 14.2.1.5.7

5.1.2.8. The proportion of patients with DDI ≤ 0.5 UI/kg b.w. (week 52, 104)

Remission was defined as DDI <0.5 U/kg/day and HbA1c <6.5%. The p-values obtained in permutation exact tests show that there was a statistically significantly higher proportion of patients in remission in the Tregs + anti-CD20 antibody rituximab group than in the control group at 3 months (p=0.0017), 6 months p=0.0029), 9 months (p=0.0194), and 21 months (p=0.0421) but not at 18 (p=0.0626) or 24 months (p=0.2333). There was no statistically significant difference between the Tregs group and the control group at any timepoint, and the proportion of patients in remission was statistically significantly higher in the Tregs + anti-CD20 antibody rituximab group than in the Tregs group at 6 months (p=0.0101) (Table 5-16). These results suggest that the combination therapy may be better in keeping patients in this population in remission.

Results are listed by patient and visit (Listing 16.2.6.9) and were analyzed using the exact permutation test in a treatment x remission (y/n) contingency table to obtain p-values for all three treatment comparisons (Table 14.2.2.8.1).

Table 5-16: Patients in Remission – Contingency Table (ITT Set)

Visit	Comparison	Proportion of patients in remission Test [%]	Proportion of patients in remission Reference [%]	p-value*
3 m	Tregs+anti-CD20 Rituximab vs Control	100.0	40.0	0.0017*
	Tregs vs Control	72.7	40.0	0.1482
	Tregs vs Tregs+anti-CD20 Rituximab	72.7	100.0	0.1676
6 m	Tregs+anti-CD20 Rituximab vs Control	91.7	27.3	0.0029*
	Tregs vs Control	38.5	27.3	0.8405
	Tregs vs Tregs+anti-CD20 Rituximab	38.5	91.7	0.0101*
9 m	Tregs+anti-CD20 Rituximab vs Control	66.7	12.5	0.0194*
	Tregs vs Control	27.3	12.5	0.8171
	Tregs vs Tregs+anti-CD20 Rituximab	27.3	66.7	0.0588
12 m	Tregs+anti-CD20 Rituximab vs Control	54.5	20.0	0.1187
	Tregs vs Control	30.0	20.0	0.8193
	Tregs vs Tregs+anti-CD20 Rituximab	30.0	54.5	0.2679
15 m	Tregs+anti-CD20 Rituximab vs Control	54.5	22.2	0.1763
	Tregs vs Control	36.4	22.2	0.6547
	Tregs vs Tregs+anti-CD20 Rituximab	36.4	54.5	0.5198
18 m	Tregs+anti-CD20 Rituximab vs Control	50.0	10.0	0.0616
	Tregs vs Control	25.0	10.0	0.6464
	Tregs vs Tregs+anti-CD20 Rituximab	25.0	50.0	0.2705
21 m	Tregs+anti-CD20 Rituximab vs Control	45.5	0.0	0.0421*
	Tregs vs Control	30.0	0.0	0.2057
	Tregs vs Tregs+anti-CD20 Rituximab	30.0	45.5	0.4734
24 m	Tregs+anti-CD20 Rituximab vs Control	33.3	10.0	0.2333
	Tregs vs Control	27.3	10.0	0.4581
	Tregs vs Tregs+anti-CD20 Rituximab	27.3	33.3	0.8137

Abbreviations: Tregs: T regulatory cells, DDI: daily insulin dose per kg body weight; ITT: intention-to-treat
Note(s): *indicates a significant difference (these values are boxed). p-value is calculated in a permutation exact test.
Visits 3m, 6m, 9m, 12m, 15m, 18m, 21m and 24m are included in the analysis.
Remission is defined as DDI < 0.5 U/kg/day and HbA1c < 6.5%.
Each time point was assessed independently. Hence patients can be observed in remission at a later timepoint even when they had a loss of remission in the Kaplan Meier analysis before due to the fact that their glucose levels had been regulated in between.
Source: Table 14.2.2.8.1, Listing 16.2.6.9

5.1.2.9. Insulin independent patients

The only statistically significant difference among the three groups in the proportion of patients with insulin-independent status was at month 6, when there was a statistically significant difference between the Tregs+anti-CD20 antibody rituximab group and the Tregs group (p=0.0308, Table 5-17). However, a survival analysis of time-to-first loss of insulin independence showed that first loss tended to occur later in the Tregs+anti-CD20 rituximab group than in both the Tregs and Control groups (Table 5-18).

Insulin-independent patients (DDI = 0 IU/kg) are listed by patient and visit for each of the follow-up visits (Listing 16.2.6.10) and were analyzed using the exact permutation test on a treatment*insulin independence (y/n) contingency table to obtain p-values for all three treatment comparisons (Table 5-17 and Table 14.2.2.9.1.2).

A survival analysis showing the time to the first insulin injection (first loss of insulin independence, starting from month 3) is shown in Table 5-18 (Table 14.2.2.9.2.2).

A Kaplan-Meier plot of the proportions of patients with insulin independence is displayed with treatments overlaid (Figure 5-18: Kaplan-Meier-Plot of First Loss of Insulin Independence (DDI = 0 U/kg/day) (ITT Set)) for the corresponding months starting from the first follow-up (month 3).

Table 5-17: Insulin Independent Patients (DDI = 0 U/kg/day) – Contingency Table (ITT Set)

Visit	Comparison	Proportion of patients in remission Test [%]	Proportion of patients in remission Reference [%]	p-value*
3 m	Tregs+anti-CD20 Rituximab vs Control	25.0	0.0	0.1032
	Tregs vs Control	8.3	0.0	0.7545
	Tregs vs Tregs+anti-CD20 Rituximab	8.3	25.0	0.3582
6 m	Tregs+anti-CD20 Rituximab vs Control	25.0	0.0	0.0539
	Tregs vs Control	0.0	0.0	1.0000
	Tregs vs Tregs+anti-CD20 Rituximab	0.0	25.0	0.0308*
9 m	Tregs+anti-CD20 Rituximab vs Control	16.7	0.0	0.1895
	Tregs vs Control	0.0	0.0	1.0000
	Tregs vs Tregs+anti-CD20 Rituximab	0.0	16.7	0.2661
12 m	Tregs+anti-CD20 Rituximab vs Control	9.1	0.0	0.6364
	Tregs vs Control	0.0	0.0	1.0000
	Tregs vs Tregs+anti-CD20 Rituximab	0.0	9.1	0.3333
15 m	Tregs+anti-CD20 Rituximab vs Control	0.0	0.0	1.0000
	Tregs vs Control	0.0	0.0	1.0000
	Tregs vs Tregs+anti-CD20 Rituximab	0.0	0.0	1.0000
18 m	Tregs+anti-CD20 Rituximab vs Control	8.3	0.0	0.6471
	Tregs vs Control	0.0	0.0	1.0000
	Tregs vs Tregs+anti-CD20 Rituximab	0.0	8.3	0.7059
21 m	Tregs+anti-CD20 Rituximab vs Control	0.0	0.0	1.0000
	Tregs vs Control	0.0	0.0	1.0000
	Tregs vs Tregs+anti-CD20 Rituximab	0.0	0.0	1.0000
24 m	Tregs+anti-CD20 Rituximab vs Control	0.0	0.0	1.0000
	Tregs vs Control	0.0	0.0	1.0000
	Tregs vs Tregs+anti-CD20 Rituximab	0.0	0.0	1.0000

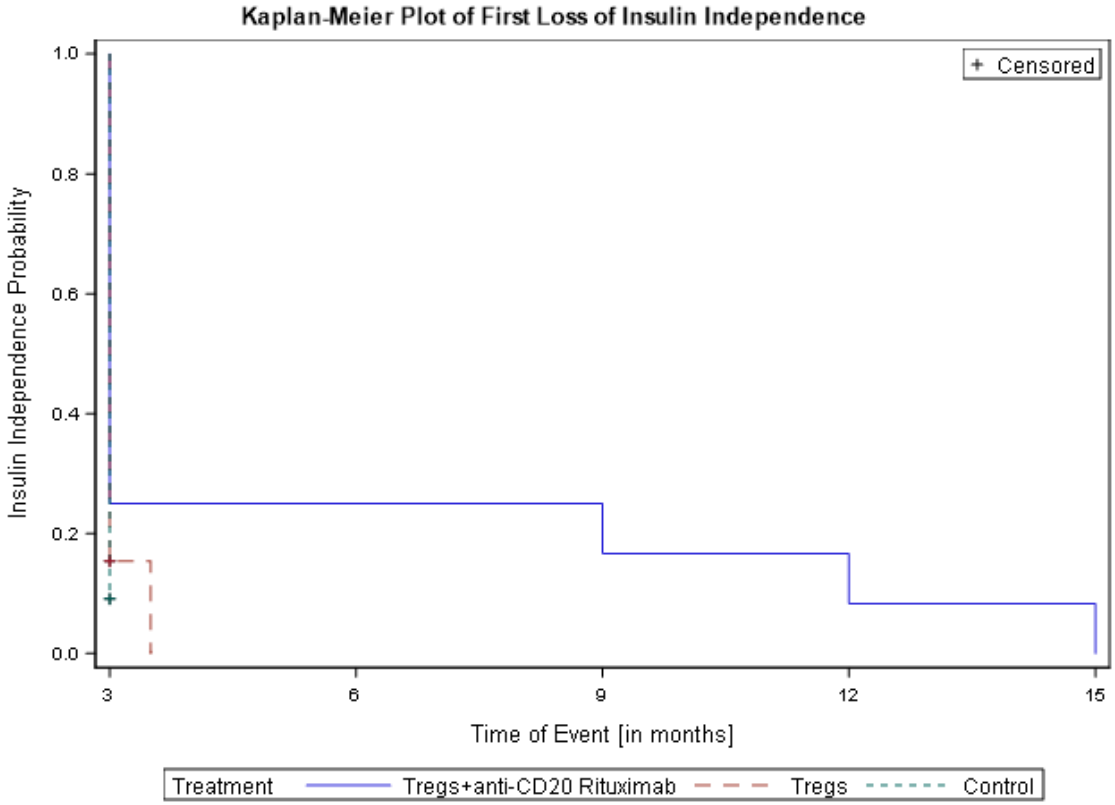
Abbreviations: Tregs: T regulatory cells, DDI: daily insulin dose per kg body weight; ITT: intention-to-treat
Note(s): * indicates a significant difference (these values are boxed). p-value is calculated in a permutation exact test.
Visits 3m, 6m, 9m, 12m, 15m, 18m, 21m and 24m are included in the analysis.
Month 3 prim is not included in the analysis, because the control group does not have data for this visit.
Each time point was assessed independently. Hence patients can be observed as insulin independent at a later timepoint even when they had a loss of insulin independence in the Kaplan Meier analysis before due to the fact that their glucose levels had been regulated in between.
Source: Table 14.2.2.9.1.2, Listing 16.2.6.10

Table 5-18: Survival Analysis of Time to First Loss of Insulin Independence (DDI= 0 U/kg/day) Starting at the 3-month Visit (ITT Set)

	Tregs+anti-CD20 Rituximab (N=12)				Tregs (N=13)				Control (N=11)			
Visit	At Risk	Event	Censored	Survival	At Risk	Event	Censored	Survival	At Risk	Event	Censored	Survival
3-m	12	9	0	0.250	13	11	1	0.154	11	10	1	0.091
3-prim					1	1	0	0.000				
9-m	3	1	0	0.167								
12-m	2	1	0	0.083								
15-m	1	1	0	0.000								

Abbreviations: Tregs: T regulatory cells, DDI: daily insulin dose per kg body weight; ITT: intention-to-treat, m: month; N: number of treated patients
Note(s): Survival – Duration of insulin independence until first loss.
This table shows the results of a survival analysis with the insulin independency status at the month 3 visit as the starting point.
Visits 3m, 3m prim, 9m, 12m, and 15m are included in the analysis.
The control group does not have Month 3 prim as a scheduled visit, whereas the active treatment groups do.
If the time to first loss of insulin independency status of a patient is not known for a scheduled visit, the patient will be censored from that visit onward.
Source: Table 14.2.2.9.2.2, Listing 16.2.6.10.2

Figure 5-18: Kaplan-Meier-Plot of First Loss of Insulin Independence (DDI = 0 U/kg/day) (ITT Set)



Abbreviations: Tregs: T regulatory cells, ITT: intention-to-treat
Note(s): Survival - Duration of insulin independence until first loss. This table shows the results of a survival analysis with the insulin independency status at the month 3 visit as the starting point.
Visits 3m, 3m prim, 9m, 12m, and 15m are included in the analysis.
The control group does not have Month 3 prim as a scheduled visit, whereas the active treatment groups do.
If the insulin independency status of a patient is not known for a scheduled visit, the patient will be censored from that visit onward.
Source: Figure 14.2.2.9.3.2

5.2. Safety

The database lock for this study was 6-Aug-2020.

The Safety Population comprised 36 patients which consisted of all randomized patients.

Safety analyses were pre-specified in the protocol (Appendix 16.1.1) and the SAP (Appendix 16.1.9).

5.2.1. Adverse Events (AEs)

Adverse events (AEs) reported during the course of the study are provided in Appendix 16.2 and Listings 16.2.7.1 and 16.2.7.2, summarized in Tables 14.3.1.1-14.3.1.6, and presented and discussed in the sections below.

For AEs in this study, the causal relationship to treatment was assessed by the investigator as related, possibly related, unlikely to be related, unrelated, or impossible to determine. For the purpose of describing and analyzing the AEs, all events which were assessed by the

investigator as related or possibly related are treated as causally related to treatment. Events which were assessed by the investigator as unlikely to be related, unrelated or impossible to determine are treated as not causally related to treatment.

5.2.1.1. Brief summary of adverse events

An overview of AEs, including the distributions of the different AE categories across all groups, is provided in Table 5-19.

In total, 156 AEs were reported in 31 patients (86.1%) across all groups. In the Tregs + CD20 antibody rituximab group, 76 AEs were reported in 12 patients (100.0%), and in the Tregs + placebo group, 28 AEs were reported in 9 patients (69.2%), resulting in a total of 104 AEs in 21 patients (84.0%) receiving active treatment. In the control group, 52 AEs were reported in 10 patients (90.9%).

AEs causally related to treatment were reported in patients receiving Tregs + CD20 antibody rituximab (59 related AEs in 12 patients, corresponding to 100.0% of patients treated with Tregs + CD20 antibody rituximab), and patients receiving Tregs + placebo (10 related AEs in 8 patients, corresponding to 61.5% of patients treated with Tregs + placebo) for a total of 69 causally related AEs in 20 patients (80.0%) receiving active treatment. No causally related AEs were reported in the control group.

All reported AEs were of mild (132 mild AEs in 29 patients, corresponding to 80.6% of patients across all groups) to moderate severity (24 moderate AEs in 8 patients, corresponding to 22.2% of patients across all groups), and no severe AEs were reported in any of the patients. In the Tregs + CD20 antibody rituximab group, 66 mild AEs were reported in 12 patients (100.0%) and 10 moderate AEs in 2 patients (16.7%), whereas in the Tregs + placebo group, 18 mild AEs were reported in 8 patients (61.5%) and 10 moderate AEs in 4 patients (30.8%), resulting in a total of 84 mild AEs in 20 (80.0%) and 20 moderate AEs in 6 (24.0%) patients receiving active treatment. In the control group, 48 mild AEs were reported in 9 patients (81.8%) and 4 moderate AEs in 2 patients (18.2%).

No serious AEs (SAEs) or deaths were reported during the study.

No AEs leading to withdrawal of study treatment were reported in any of the patients.

Table 5-19: Adverse Events – Summary Table by Treatment Group (Safety Set)

Category	Tregs+antiCD20 Rituximab (N=12) n (%) E	Tregs (N=13) n (%) E	Control (N=11) n (%) E	Total (N=36) n (%) E
Any AE	12 (100) 76	9 (69.2) 28	10 (90.9) 52	31 (86.1) 156
Any SAE	0	0	0	0
Death	0	0	0	0
AE Leading to Withdrawal of Study Drug	0	0	0	0
SAE Leading to Withdrawal of Study Drug	0	0	0	0
AE by Relationship				
Any Related AE	12 (100) 59	8 (61.5) 10	0	20 (55.6) 69
Any Unrelated AE	7 (58.3) 17	5 (38.5) 18	10 (90.9) 52	22 (61.1) 87
AEs by Severity				
Mild	12 (100) 66	8 (61.5) 18	9 (81.8) 48	29 (80.6) 132
Moderate	2 (16.7) 10	4 (30.8) 10	2 (18.2) 4	8 (22.2) 24
Severe	0	0	0	0
Patients with at least one SAE	0	0	0	0

Abbreviation(s): Tregs: T regulatory cells, n: number of patients having an adverse event, N: number of patients at risk, E: number of events, AE: Adverse event, SAE: Serious adverse event
Note(s): % - n/N*100, where N is the number of patients in each group.
Related AE: possibly related or related AE; Unrelated AE: impossible, unlikely related or unrelated AE.
Source: Table 14.3.1.1, Listing 16.2.7.1, 16.2.7.2

5.2.1.2. Display of AEs

A complete overview of AEs by MedDRA System Organ Class (SOC), including MedDRA Preferred Terms for AEs reported in two or more patients, is provided in Table 5-20 (Table 14.3.1.2). AEs are listed individually in Listing 16.2.7.1.

Across all groups, of 156 total AEs reported in 31 patients (86.1% of all patients), the most frequently reported AEs were 10 events categorized as respiratory tract infection, including 10 (27.8%) out of all patients, 4 (30.8%) Tregs + placebo patients, 3 (25.0%) Tregs + CD20 antibody rituximab patients, and 3 (27.3%) control patients. Of these 10 events, 3 events that occurred in 3 (25.0%) Tregs + CD20 antibody rituximab patients and 2 events that occurred in 2 (15.4%) Tregs + placebo patients were reported as related to treatment. The next most frequently reported AEs were 8 events categorized as abdominal pain that were reported in 5 patients (13.9% of all patients); these included 4 patients in the Tregs + CD20 antibody rituximab group (33.3% of this patient group) and 1 patient in the Tregs + placebo group (7.7% of this patient group). Seven (7) events of iron deficiency were reported in 5 patients (13.9% of all patients), including 1 patient in the Tregs + CD20 antibody rituximab group (8.3% of this patient group) and 4 patients in the control group (36.4% of this patient group). Six (6) events of headache were reported in 5 patients (13.9% of all patients), including 2 patients in the Tregs + CD20 antibody rituximab group (16.7% of patients in this group), 1 patient in the Tregs + placebo group (7.7% of patients in this group), and 2 patients in the control group (18.2% of these patients). Among these events, the following were reported as related to study treatment: abdominal pain in 4 patients in the Tregs + CD20 antibody rituximab group (33.3% of this patient group), iron deficiency in 1 patient in the Tregs + CD20 antibody rituximab group (8.3% of this patient group), and headache in 2 patients in the

Tregs + CD20 antibody rituximab group (16.7% of this patient group) and 1 patient in the Tregs + placebo group (7.7% of this patient group).

Table 5-20: Adverse Events – Frequency Table by Treatment Group (Safety Set)

System Organ Class Preferred Term	Tregs+anti-CD20 Rituximab (N=12) n (%) E	Tregs (N=13) n (%) E	Control (N=11) n (%) E
Any AE	12 (100) 76	9 (69.2) 28	10 (90.9) 52
Infections and infestations	7 (58.3) 11	6 (46.2) 9	4 (36.4) 5
Respiratory tract infection	3 (25.0) 3	4 (30.8) 4	3 (27.3) 3
Infection	1 (8.3) 1	1 (7.7) 1	0
Respiratory tract infection bacterial	0	1 (7.7) 1	1 (9.1) 1
Upper respiratory tract infection	1 (8.3) 1	0	1 (9.1) 1
Metabolism and nutrition disorders	3 (25.0) 8	2 (15.4) 3	6 (54.5) 11
Iron deficiency	1 (8.3) 1	0	4 (36.4) 6
Hyperglycaemia	2 (16.7) 2	0	2 (18.2) 2
Hypoglycaemia	1 (8.3) 2	1 (7.7) 1	2 (18.2) 2
Diabetes mellitus inadequate control	1 (8.3) 1	1 (7.7) 1	1 (9.1) 1
Investigations	2 (16.7) 2	1 (7.7) 1	5 (45.5) 14
Protein urine present	0	0	4 (36.4) 5
Blood urine present	0	0	3 (27.3) 3
White blood cells urine positive	0	0	3 (27.3) 4
Blood and lymphatic system disorders	4 (33.3) 4	0	3 (27.3) 3
Eosinophilia	1 (8.3) 1	0	2 (18.2) 2
Neutropenia	2 (16.7) 2	0	0
Gastrointestinal disorders	4 (33.3) 14	1 (7.7) 5	1 (9.1) 1
Abdominal pain	4 (33.3) 6	1 (7.7) 2	0
Nausea	3 (25.0) 4	0	0
Diarrhoea	1 (8.3) 1	1 (7.7) 1	0
Vomiting	2 (16.7) 3	0	0
General disorders and administration site conditions	4 (33.3) 5	2 (15.4) 2	0
Asthenia	2 (16.7) 2	1 (7.7) 1	0
Vessel puncture site haematoma	1 (8.3) 1	1 (7.7) 1	0
Nervous system disorders	2 (16.7) 5	1 (7.7) 1	3 (27.3) 3
Headache	2 (16.7) 3	1 (7.7) 1	2 (18.2) 2
Surgical and medical procedures	1 (8.3) 1	2 (15.4) 2	3 (27.3) 3
Antibiotic therapy	1 (8.3) 1	1 (7.7) 1	1 (9.1) 1
Symptomatic treatment	0	0	2 (18.2) 2
Psychiatric disorders	2 (16.7) 7	1 (7.7) 4	2 (18.2) 6
Adjustment disorder	2 (16.7) 2	1 (7.7) 2	1 (9.1) 1
Emotional disorder	1 (8.3) 1	1 (7.7) 1	2 (18.2) 3
Behaviour disorder	0	0	1 (9.1) 2
Respiratory, thoracic and mediastinal disorders	3 (25.0) 5	0	0
Oropharyngeal pain	2 (16.7) 2	0	0
Cardiac disorders	2 (16.7) 3	0	0
Tachycardia	2 (16.7) 3	0	0
Injury, poisoning and procedural complications	1 (8.3) 1	0	1 (9.1) 1
Social circumstances	2 (16.7) 2	0	0

Abbreviation(s): Tregs: T regulatory cells, n: number of patients having an adverse event, N: number of patients at risk, E: number of events, AE: Adverse event.
Note(s): % - (n/N) 100, where N is the number of patients in each group. Note that only entries with n≥2 in the Total column are included in this table. In summarizing n(%), if a patient has multiple AEs for the same System Organ Class (SOC) or Preferred Term (PT), the patient is counted only once for the given SOC and PT
according to MedDRA dictionary Version 23.0.
Source: Table 14.3.1.2, Listing 16.2.7.1

5.2.1.3. Analysis of AEs

AE frequencies by relationship to treatment are displayed in Table 14.3.1.3 and summarized in Table 5-21. AE frequencies by severity are displayed in Table 14.3.1.4. AEs are listed individually in Listing 16.2.7.1.

All AEs which were assessed as related or possibly related by the investigator are treated as causally related to treatment. None of the related AEs was serious.

In the Tregs + CD20 antibody rituximab group, all patients (12 patients, 100%) reported at least one related AE. The most frequently reported related AEs in this group were 6 events of abdominal pain in 4 patients (33.3% of Tregs + CD20 antibody rituximab patients) and 4 events of nausea in 3 patients (25.0% of Tregs + CD20 antibody rituximab patients). Three events each of tachycardia, headache and vomiting as well as 2 events each of oropharyngeal pain, asthenia, hyperglycaemia and neutropenia were reported as related in 2 patients (16.7% of Tregs + CD20 antibody rituximab patients) each. Other AEs were reported as related only once each (8.3% of Tregs + CD20 antibody rituximab patients) in the Tregs + CD20 antibody rituximab group.

In the Tregs + placebo group, 8 patients (61.5% of Tregs + placebo patients) reported at least one related AE. No AE was reported in more than one patient in this group, so that all related AEs were reported only in single patients (1 patient or 7.7% of Tregs + placebo patients).

There were no AEs of severe severity.

Table 5-21: Adverse Events – Frequency Table by Treatment Group and Relationship to Study Drug (Safety Set)

System Organ Class Preferred Term	Tregs+anti-CD20 Rituximab (N=12) n (%) E	Tregs (N=13) n (%) E	Total Active (N=25) n (%) E
Any AE	12 (100) 59	8 (61.5) 10	20 (80.0) 69
Infections and infestations	6 (50.0) 8	4 (30.8) 5	10 (40.0) 13
Respiratory tract infection	1 (8.3) 1	1 (7.7) 1	2 (8.0) 2
Infection	1 (8.3) 1	1 (7.7) 1	2 (8.0) 2
Respiratory tract infection bacterial	0	1 (7.7) 1	1 (4.0) 1
Upper respiratory tract infection	1 (8.3) 1	0	1 (4.0) 1
Herpes zoster	0	1 (7.7) 1	1 (4.0) 1
Influenza	1 (8.3) 1	0	1 (4.0) 1
Moraxella infection	1 (8.3) 1	0	1 (4.0) 1
Mumps	0	1 (7.7) 1	1 (4.0) 1
Pharyngitis	1 (8.3) 1	0	1 (4.0) 1
Respiratory tract infection viral	1 (8.3) 1	0	1 (4.0) 1
Viral rash	1 (8.3) 1	0	1 (4.0) 1
Metabolism and nutrition disorders	3 (25.0) 5	1 (7.7) 1	4 (16.0) 6
Hyperglycaemia	2 (16.7) 2	0	2 (8.0) 2
Hypoglycaemia	1 (8.3) 2	1 (7.7) 1	2 (8.0) 3
Hyperinsulinism	1 (8.3) 1	0	1 (4.0) 1
Investigations	1 (8.3) 1	0	1 (4.0) 1
Inflammatory marker increased	1 (8.3) 1	0	1 (4.0) 1
Blood and lymphatic system disorders	3 (25.0) 3	0	3 (12.0) 3
Neutropenia	2 (16.7) 2	0	2 (8.0) 2
Iron deficiency anaemia	1 (8.3) 1	0	1 (4.0) 1
Gastrointestinal disorders	4 (33.3) 14	0	4 (16.0) 14
Abdominal pain	4 (33.3) 6	0	4 (16.0) 6
Nausea	3 (25.0) 4	0	3 (12.0) 4
Diarrhoea	1 (8.3) 1	0	1 (4.0) 1
Vomiting	2 (16.7) 3	0	2 (8.0) 3
General disorders and administration site conditions	4 (33.3) 5	2 (15.4) 2	6 (24.0) 7
Asthenia	2 (16.7) 2	1 (7.7) 1	3 (12.0) 3
Vessel puncture site haematoma	1 (8.3) 1	1 (7.7) 1	2 (8.0) 2
Chills	1 (8.3) 1	0	1 (4.0) 1
Fatigue	1 (8.3) 1	0	1 (4.0) 1
Nervous system disorders	2 (16.7) 5	1 (7.7) 1	3 (12.0) 6
Headache	2 (16.7) 3	1 (7.7) 1	3 (12.0) 4
Dizziness	1 (8.3) 1	0	1 (4.0) 1
Somnolence	1 (8.3) 1	0	1 (4.0) 1
Surgical and medical procedures	1 (8.3) 1	1 (7.7) 1	2 (8.0) 2
Antibiotic therapy	1 (8.3) 1	0	1 (4.0) 1
Therapy cessation	0	1 (7.7) 1	1 (4.0) 1
Respiratory, thoracic and mediastinal disorders	2 (16.7) 4	0	2 (8.0) 4
Oropharyngeal pain	2 (16.7) 2	0	2 (8.0) 2
Dry throat	1 (8.3) 1	0	1 (4.0) 1
Dyspnoea	1 (8.3) 1	0	1 (4.0) 1
Skin and subcutaneous tissue disorders	2 (16.7) 5	0	2 (8.0) 5
Diffuse alopecia	1 (8.3) 2	0	1 (4.0) 2
Hyperhidrosis	1 (8.3) 1	0	1 (4.0) 1
Rash	1 (8.3) 1	0	1 (4.0) 1
Skin hyperpigmentation	1 (8.3) 1	0	1 (4.0) 1
Cardiac disorders	2 (16.7) 3	0	2 (8.0) 3
Tachycardia	2 (16.7) 3	0	2 (8.0) 3
Injury, poisoning and procedural complications	1 (8.3) 1	0	1 (4.0) 1
Infusion related reaction	1 (8.3) 1	0	1 (4.0) 1
Social circumstances	1 (8.3) 1	0	1 (4.0) 1
Death of relative	1 (8.3) 1	0	1 (4.0) 1
Musculoskeletal and connective tissue disorders	1 (8.3) 1	0	1 (4.0) 1
Muscle twitching	1 (8.3) 1	0	1 (4.0) 1
Product issues	1 (8.3) 1	0	1 (4.0) 1
Device breakage	1 (8.3) 1	0	1 (4.0) 1
Vascular disorders	1 (8.3) 1	0	1 (4.0) 1

Venous thrombosis limb	1 (8.3) 1	0	1 (4.0) 1	

Abbreviations: n: number of patients having an adverse event, N: number of patients at risk, E: number of events, AE: Adverse event, Tregs: T regulatory cells.
Note(s): %=(n/N) *100, where N is the number of patients in each group. Related AE: possibly related or related AE. System Organ Class and Preferred Term according to MedDRA dictionary Version 23.0.
Source: Table 14.3.1.3, Listing 16.2.7.1

5.2.1.4. Listing of AEs by patients

A by-patient listing of AEs is provided in Appendix 16.2, Listing 16.2.7.1.

5.2.2. Deaths, other serious AEs and other significant AEs

5.2.2.1. Deaths, serious AEs and AEs leading to withdrawal of study treatment

SAEs and AEs leading to withdrawal of a study treatment are displayed in Tables 14.3.1.5 and 14.3.1.6, respectively. A by-patient listing of SAEs and AEs that led to withdrawal of a study treatment is provided in Appendix 16.2, Listing 16.2.7.2.

No serious AEs (SAEs) or deaths were reported during the study.

No AEs leading to withdrawal of a study treatment were reported in any of the patients.

5.2.2.2. AEs of special importance

Four groups of adverse events of special importance (AESIs) were defined in section 6.3 of the protocol (Appendix 16.1.1) for this study, these being AEs related with blood collection and administration of the Tregs, Treg product contamination, AEs related with the immunosuppressive activity of Tregs, and adverse effects related with administration of rituximab antibody (antiCD20). For all four groups, specific AEs or broader types of AEs which should be considered as belonging to the groups were outlined in the protocol. AESIs that occurred in the groups receiving Tregs with rituximab or placebo is presented in Table 5-22.

Of these, the most common during the study were various events in the infections and infestations SOC, 11 of which occurred in 7 patients of the Tregs + CD20 antibody rituximab group (58.3% of this patient group), compared to 9 events from this SOC in 6 patients of the Tregs + placebo group (46.2% of this patient group) and 5 events in 4 patients of the control group (36.4% of this patient group). Most of the reported infections were of mild severity, but 2 events (one each of upper respiratory tract infection and influenza) both in the same patient in the Tregs + CD20 antibody rituximab group (8.3% of this patient group) as well as 2 events (one each of herpes zoster and mumps) in 2 patients of the Tregs + placebo group (15.4% of this patient group) were of moderate severity. In the Tregs + CD20 antibody rituximab group, 8 of the reported infections occurring in 6 patients (50.0% of this patient group) were reported as causally related to study treatment, including both events of moderate severity. In the Tregs + placebo group, 5 of the reported infections in 4 patients (30.8% of this patient group) were reported as causally related to study treatment, also including both events of moderate severity. Infections were defined as AESIs in both the group of AEs related with the

immunosuppressive activity of Tregs and the group of adverse effects related with administration of rituximab antibody (antiCD20) in the protocol.

Other than infections, there were isolated reports of nausea (4 events in 3 patients or 25.0% of the Tregs + CD20 antibody rituximab group), headache (3 events in 2 patients or 16.7% of the Tregs + CD20 antibody rituximab group, and 1 event in 1 patient or 7.7% of the Tregs + placebo group), asthenia (2 events in 2 patients or 16.7% of the Tregs + CD20 antibody rituximab group, and 1 event in 1 patient or 7.7% of the Tregs + placebo group), vomiting (3 events in 2 patients or 16.7% of the Tregs + CD20 antibody rituximab group), neutropenia (2 events in 2 patients or 16.7% of the Tregs + CD20 antibody rituximab group) and diffuse alopecia (2 events in 1 patient or 8.3% of the Tregs + CD20 antibody rituximab group), as well as chills, somnolence, dyspnoea, rash and infusion related reaction (all occurring as 1 event each in 1 patient or 8.3% of the Tregs + CD20 antibody rituximab group), and thrombocytopenia and urticaria (both as 1 event in 1 patient or 9.1% of the control group). All of these events were of mild severity. With the exception of the events occurring in the control group, all of them were reported as causally related to study treatment. All of these were defined as AESIs under the group of adverse effects related with administration of rituximab antibody (antiCD20) in the protocol, with chills additionally also defined as an AESI in the group of AEs related with blood collection and administration of the Tregs.

Finally, vessel puncture site haematoma was reported in 1 patient each of the Tregs + CD20 antibody rituximab group (8.3% of this group) and the Tregs + placebo group (7.7% of this group). The event in the Tregs + CD20 antibody rituximab group was of mild severity, whereas the event in the Tregs + placebo group was of moderate severity. The events were reported as causally related to study treatment. The protocol defined this as an AESI in the group of AEs related with blood collection and administration of the Tregs.

No other AESIs were reported in any patients during the study. In particular, there were no reports of malignant neoplasms (defined as AESIs in both the group of AEs related with the immunosuppressive activity of Tregs and the group of adverse effects related with administration of rituximab antibody (anti-CD20) due to both treatments immunomodulatory effects), and no reports of Treg product contamination.

Table 5-22: Adverse Events of Special Interest - Frequency Table by Treatment Group (Safety Set)

Preferred Term	Tregs+anti-CD20 Rituximab (N=12) n (%) E	Tregs (N=13) n (%) E	Total Active (N=25) n (%) E
All AESIs	9 (75.0%) 23	3 (23.1%) 4	12 (48.0%) 27
Infection	1 (8.3) 1	1 (7.7) 1	2 (8.0) 2
Neutropenia	2 (16.7) 2	0	2 (8.0) 2
Nausea	3 (25.0) 4	0	3 (12.0) 4
Vomiting	2 (16.7) 3	0	2 (8.0) 3
Asthenia	2 (16.7) 2	1 (7.7) 1	3 (12.0) 3
Vessel puncture site haematoma	1 (8.3) 1	1 (7.7) 1	2 (8.0) 2
Chills	1 (8.3) 1	0	1 (4.0) 1
Headache	2 (16.7) 3	1 (7.7) 1	3 (12.0) 4
Somnolence	1 (8.3) 1	0	1 (4.0) 1
Dyspnoea	1 (8.3) 1	0	1 (4.0) 1
Diffuse alopecia	1 (8.3) 2	0	1 (4.0) 2
Rash	1 (8.3) 1	0	1 (4.0) 1
Infusion related reaction	1 (8.3) 1	0	1 (4.0) 1

Abbreviations: n: number of patients having an adverse event, N: number of patients at risk, E: number of events, AE: Adverse event, Tregs: T regulatory cells, AESI: adverse event of special interest.
Note(s): %=(n/N) *100, where N is the number of patients in each group. Preferred Term according to MedDRA dictionary Version 23.0.
Source: Table 14.3.1.3, Listing 16.2.7.1

5.2.3. Clinical laboratory evaluation

Descriptive statistics of safety laboratory values, immunophenotype B lymphocytes and Tregs, and autoantibodies are provided in Tables 14.3.4.1, 14.3.4.2.1, and 14.3.4.3.1, respectively. By-patient listings of laboratory parameters, immunophenotypes, and autoantibodies are provided in Listings 16.2.8.1, 16.2.8.3, and 16.2.8.4, respectively.

Out-of-range results of hematology parameters were reported in all patients in both the active treatment groups and the control group. The only other out-of-range results were isolated increased urine pH in 2 patients (1 patient each in the Tregs + CD20 antibody rituximab group and the control group) and isolated increased specific urine specific gravity in 1 patient from the Tregs + placebo group.

Clinically significant laboratory results were reported as AEs.

5.3. Pharmacokinetics

An analysis of the pharmacokinetics of the study intervention will be included in another report.

5.4. Pharmacodynamics

Not applicable.

5.5. Genetics

Not applicable.

5.6. Biomarkers

An analysis of the relationship of biomarkers to the study intervention will be included in another report.

5.7. Immunogenicity

Descriptive statistics for Immunophenotype (B lymphocytes and T lymphocytes) are shown by treatment for the safety set in Table 14.3.4.2.1 (Table 5-23). Mean concentration time plots of immunophenotypes (ITT set) are shown in Figure 14.3.4.2.2.

Table 5-23: Immunophenotype. B Lymphocytes and Tregs – Descriptive Statistics by Treatment (Safety Set)

Study Day	Tregs+antiCD20 Rituximab (N=12) (n) mean±SD (range)	Tregs (N=13) mean±SD (range)	Control (N=11) mean±SD (range)	Total (N=36) mean±SD (range)
	OV	OV	OV	OV
Parameter: Blood B-lymphocytes (%)				
Day 0	(12) 8.13±6.415	(12) 5.94±3.32	(10) 9.63±6.257	(34) 7.80±5.514
Day 14	(12) 6.10±3.777	(10) 9.66±3.804	(0)	(22) 7.72±4.119
Month 3	(4) 1.15±2.034	(13) 9.29±5.944	(7) 6.84±5.719	(24) 7.22±6.032
Month 6	(6) 6.33±11.782	(13) 8.44±6.940	(8) 10.36±9.103	(27) 8.54±8.566
Month 12	(11) 5.46±3.671	(9) 7.42±4.512	(7) 8.89±8.591	(27) 7.00±5.523
Month 18	(8) 6.88±2.174	(10) 7.52±3.513	(8) 2.73±1.226	(26) 5.85±3.281
Month 24	(11) 6.9±5.329	(9) 5.31±3.151	(9) 5.87±6.232	(29) 6.09±4.954
Parameter: Blood T-lymphocytes (%)				
Day 0	(12) 5.961±2.325	(13) 5.044±1.922	(10) 5.684±3.284	(35) 5.541±2.464
Day 14	(12) 4.555±1.730	(12) 5.573±2.230	(0)	(24) 5.064±2.020
Day 36	(12) 5.246±2.336	(12) 5.531±2.494	(6) 6.690±3.289	(30) 5.649±2.5568
Month 3	(12) 6.364±2.833	(13) 6.385±1.991	(9) 5.623±3.698	(34) 6.176±2.747
Month 6	(11) 6.939±3.107	(13) 4.702±2.130	(9) 5.030±2.163	(33) 5.537±2.631
Month 12	(11) 7.183±2.689	(11) 5.985±1.661	(8) 4.871±2.473	(30 6.127±2.406
Month 18	(10) 5.828±2.022	(12) 6.027±1.857	(9) 5.484±2.351	(31) 5.805±2.004

Abbreviations: Tregs: T regulatory cells, n: number of patients included in the analysis, N: number of treated patients, OV: Observed value, SD: standard deviation
Note(s): Baseline is defined as last value of assessment prior to first drug administration.
Source: Table 14.3.4.2.1, Listing 16.2.8.3

5.8. Health Economics OR Medical Resource Utilization and Health Economics

Not applicable

5.9. Other Analyses/Results: Autoantibodies

A listing for autoantibodies in the randomization set is provided in Listing 16.2.8.4.Descriptive statistics for autoantibodies in the safety set are shown in Table 14.3.4.3.1 (Table 5-24). Mean concentration time plots of autoantibodies (ITT set) are shown in Figure 14.3.4.3.2.

Table 5-24: Autoantibodies – Descriptive Statistics (Safety Set)

Study Day	Tregs+antiCD20 Rituximab (N=12) (n) mean±SD (range)	Tregs (N=13) mean±SD (range)	Control (N=11) mean±SD (range)	Total (N=36) mean±SD (range)
	OV	OV	OV	OV
Parameter: Blood Glutamic Acid Decarboxylase Antibody (IU/mL)				
Recruitment	(12) 380.9±594.6	(12) 856.6±936.5	(11) 744.3±768.3	(35) 658.2±784.3
Day A	(1) 21.28±21.28	(0)	(0)	(1) 21.28±21.28
Day 14	(11) 407.3±640.6	(13) 804.4±937.9	(9) 899.9±892.5	(33) 698.1±838.1
Month 3	(11) 214.9±267.6	(12) 724.7±944.5	(10) 849.11±873.3	(33) 592.5±787.2
Month 6	(12) 324.8±563.2	(13) 675.9±921.8	(11) 690.3±854.1	(36) 563.3±792.9
Month 12	(11) 339.7±590.1	(12) 648.6±880.3	(10) 765.1±895.4	(33) 581.0±795.6
Month `18	(12) 178.8±370.4	(12) 677.2±927.3	(9) 539.4±838.4	(33) 458.4±753.4
Month 24	(12) 254.6±563.1	(10) 629.0±946.8	(10) 662.1±877.6	(32) 499.0±795.9
Parameter: Blood Islet Cell Antibody (titer)				
Recruitment	(12) 125.0±185.3	(13) 109.2±180.5	(11) 50.9±47.63	(36) 96.7±153.6
Day 14	(11) 70.0±97.49	(13) 66.9±95.86	(9) 182.2±264.5	(33) 99.4±163.0
Month 3	(11) 56.4±92.44	(12) 45.0±61.57	(10) 216.0±392.9	(33) 100.6±231.1
Month 6	(12) 71.7±119.8	(13) 44.6±58.97	(11) 92.7±184.2	(36) 68.3±125.7
Month 12	(11) 35.5±51.65	(12) 100.0±192.6	(10) 32.0±52.66	(33) 57.9±124.1
Month 18	(12) 45.0±59.16	(12) 20.0±26.97	(9) 97.8±209.9	(33) 50.3±116.0
Month 24	(12) 70.0±99.64	(10) 38.4±57.42	(10) 52.0±65.46	(32) 54.5±76.83
Parameter: Blood Insulin Autoantibody (IU/mL)				
Recruitment	(12) 5.323±5.517	(13) 8.671±8.607	(11) 4.972±5.152	(36) 6.425±6.746
Day 14	(11) 3.813±4.530	(13) 5.028±6.007	(9) 4.296±4.958	(33) 4.423±5.135
Month 3	(11) 1.404±1.817	(12) 5.472±11.70	(10) 2.804±2.899	(33) 3.307±7.316
Month 6	(12) 2.398±3.275	(13) 1.961±2.026	(11) 1.945±1.760	(36) 2.101±2.389
Month 12	(11) 1.011±0.862	(11) 1.630±2.851	(10) 0.703±0.419	(32) 1.128±1.751
Month 18	(12) 3.704±6.783	(12) 1.431±1.744	(9) 0.997±0.514	(33) 2.139±4.290
Month 24	(12) 1.083±1.574	(10) 2.155±4.860	(10) 0.940±0.612	(32) 1.373±2.852

Abbreviation(s): Tregs: T regulatory cells, n: number of patients included in the analysis, N: number of treated patients; OV: observed value, SD: standard deviation
Note(s): Baseline is defined as last value of assessment prior to first drug administration
Source: Table 14.3.4.3.1, Listing 16.2.8.4

5.10. Summary of Evaluation of Response to Study Intervention

The results of the primary and secondary efficacy analyses strongly support the notion that both treatments delayed progression of T1DM in this patient population. Indicators of T1DM progression, including C peptide (fasted, MMTT, and glucagon test), HbA1c, glucose, and DDI, were consistently superior, often statistically significantly so, in the two treatment groups. They were also consistently superior in the combination therapy / monotherapy comparisons, with the inferiority of the monotherapy group indicated in some cases. The proportion of patients in remission was consistently better in the combination therapy group (but not the monotherapy group) than in the control group, and there was a clear trend for the time to first loss of remission to occur later in patients in the combination therapy group than in either the monotherapy group or the control group. Finally, the first loss of insulin independence also tended to occur later in the combination therapy group than in either the monotherapy group or the control group, but these differences were not significant.

No deaths, serious adverse events (SAEs), severe adverse events (AEs), or AEs leading to withdrawal of study treatment were reported during this study.

6. CONCLUSIONS

The following is a summarized list of specific key findings of this study related to the study objectives:

- No deaths, serious adverse events (SAEs), severe adverse events (AEs), or AEs leading to withdrawal of study treatment were reported during this study.
- While most of the patients in the treatment groups experienced AEs considered causally related to the administration of Tregs or rituximab, most AEs were mild and none were severe, indicating the treatments were well-tolerated.
- AUC of C peptide and C peptide levels (MMTT) were higher in both treatment groups than in the control group.
- For AUC of C peptide at 24 months (glucagon test), the monotherapy was statistically significantly inferior to the combination therapy (0.533, 90% CI 0.305-0.932).
- For C peptide levels (fasted) at the 24-month visit, the monotherapy was inferior to the combined therapy (0.553, 90% CI 0.309-0.989), whereas when analyzed at all visits, the monotherapy was statistically significantly inferior to the combination therapy at 18 (0.630, 90% CI 0.416-0.955), 21 (0.511 90% CI 0.304-0.859), and 24 (0.528 90% CI 0.314-0.889) months.
- Point estimates for HbA1c were significantly lower for the combination therapy than the control at later timepoints (3, 6, 9, 12, 21, and 24 months). Importantly, the combination therapy was superior to the monotherapy at every visit except for the 9-month visit.
- Most comparisons in glucose levels between the two treatment groups showed non-inferiority of the monotherapy, especially in year 2.
- DDI was not significantly different between the two treatment groups at any timepoint, but DDI was consistently lowest in the combination therapy group
- When analyzed over the course of the study, the proportion of patients in remission was significantly higher in the Tregs+anti-CD20 antibody rituximab group than in the control group at 3 (p=0.0017), 6 p=0.0029), 9 (p=0.0194), and 21 (p=0.0421) months but not at 18 (p=0.0626) or 24 (p=0.2333) months, but there was never a significant difference between the monotherapy and the control group. There was a clear trend in the time-to-event (time to first loss of remission) analysis for the proportion of patients in remission to decrease more slowly in the combination therapy group than in either the monotherapy group or the control group.
- The proportion of patients with insulin-independent status was significantly higher for the combination therapy than for the monotherapy at month 6 (p=0.0308), and a survival analysis of time-to-first loss of insulin independence showed that first loss tended to occur later in the Tregs+anti-CD20 rituximab group than in both the Tregs and Control group.

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