

Dose-finding safety and tolerability study of anti-CD3 monoclonal antibody oteelixizumab in new onset Type 1 diabetes

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Electronic supplementary material

Abbreviations

CPT: Cell Preparation Tube

FDA: Food and Drug Administration

ULN: Upper limit of normal

ESM Methods

Safety

Liver chemistry stopping criteria

Liver chemistry threshold stopping criteria were designed to assure patient safety and to evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance). Study treatment was stopped for a patient if any of the following liver chemistry stopping criteria were met:

1. ALT $\geq$ 3xULN and bilirubin $\geq$ 2xULN

Serum bilirubin fractionation should have been performed if testing was available. If fractionation was unavailable, urinary bilirubin was to be measured via dipstick (a measurement of direct bilirubin, which would suggest liver injury).

2. ALT $\geq$ 5xULN.
3. ALT $\geq$ 3xULN if associated with symptoms (new or worsening) believed to be related to hepatitis (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness or jaundice) or believed to be related to hypersensitivity (such as fever, rash or eosinophilia).
4. ALT $\geq$ 3xULN persists for $\geq$ 4 weeks.
5. ALT $\geq$ 3xULN and cannot be monitored weekly for 4 weeks.

Subjects with ALT $\geq$ 3xULN and $<$ 5xULN and bilirubin $<$ 2xULN, who did not exhibit hepatitis symptoms or rash, could continue study treatment as long as they could be monitored weekly for 4 weeks.

Exploratory biomarkers

Blood samples for analysis of exploratory biomarkers were collected at pre-dose baseline, pre-dose on day 6 of dosing, at week 6, and at months 3, 6 and 24. Blood was collected into sodium citrate CPT™ tubes (BD Biosciences) and PBMC fractions were separated and cryopreserved at the clinical sites, within 8 hours of collection to stabilise the samples.

For enumeration of lymphocyte subsets, cryopreserved PBMCs were thawed, washed and stained with surface antibodies, including CD3, CD8, CD45RA, KLRG1 and TIGIT, before permeabilization (FOXP3 Fix/Perm Buffer Set, BioLegend) for EOMES staining, adapted from Long et al (1); defining CD3+CD8-EOMES+KLRG1+TIGIT+ as a partially exhausted phenotype. In an additional flow cytometry panel, thawed PBMCs were stained to identify T cell subsets (Th1, Th2, Th17, Tc1, Tc2, Tc17, CD4 Treg and CD8 Treg) using surface antibodies: CD4, CD8, CD45RA, CD25, CD127, CXCR3 and CCR6. To allow subject stratification for further testing (i.e. quantification of EBV-reactive CD8 T cells, restricted to HLA-A2 positive subjects), HLA-A2 surface staining was included. Data were acquired on a FACS Canto II using FACS Diva Software and analysed using FlowJo X. Isotype controls were used to define negative population - positive gates were set to include ~1% of events in these samples.

The identification and quantification of HLA-A2 restricted EBV-reactive CD8 T cells (Latent Membrane Protein-2 426-434 CLGGLLTMV) from cryopreserved PBMCs was performed using the Diab-Q-kit, developed by Leiden University Medical Center (2). The Diab-Q-kit uses the combinatorial encoding method whereby epitope specific CD8 T cells are identified by staining with a combination of two HLA class I multimers that have the same epitope but different fluorochromes. Data were acquired and analysed on a BD LSRII using FACS Diva Software.

Immunosequencing of the CDR3 regions of human TCR β chains was performed using the ImmunoSEQ™ Assay (Adaptive Biotechnologies, Seattle, WA). For the preparation of cDNA, RNA was isolated from PAXgene Blood by Q² Solutions using Thermo-Fisher High Capacity RNA-to-cDNA kit. cDNA was amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing. Sequences were collapsed and filtered in order to identify and quantitate the absolute abundance of each unique TCR β CDR3 region for further analysis as previously described (3-5). Productive clonality was used to assess the degree of mono or oligoclonality within the TCR β repertoire. Clonality was defined as 1- Peilou's

eveness (6), and was calculated on productive rearrangements by: $1 + \frac{\sum_{i=1}^N p_i \log_2(p_i)}{\log_2(N)}$ where

p_i is the proportional abundance of rearrangement i and N is the total number of rearrangements. The productive clonality statistic was used to measure skew in the T-cell repertoire towards high frequency, expanded T-cell clones. Productive clonality values range from 0 to 1 and describe the shape of the frequency distribution: values approaching 1 indicate an increasingly asymmetric distribution in which a few clones are present at high frequencies. Statistical analysis was performed in R version 3.2.

Sequence and meta data that have been made publicly available were used for querying CDR3 amino acid sequences from EBV-reactive TCR β sequences against each repertoire and summed the frequencies of all matched clones on a per sample basis (7-26).

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ESM Table 1: Dosage cohorts

Cohort	Cumulative dose (mg)	Day 1			Day 2			Day 3			Days 4-6		
		Daily dose (mg)	Rate of infusion (mg/h)	Infusion time (h)	Daily dose (mg)	Rate of infusion (mg/h)	Infusion time (h)	Daily dose (mg)	Rate of infusion (mg/h)	Infusion time (h)	Daily dose (mg)	Rate of infusion (mg/h)	Infusion time (h)
1	9	1.5	0.167	9	1.5	0.25	6	1.5	0.5	3	1.5	1.5	1
2	18	3	0.25	12	3	0.5	6	3	1	3	3	3	1
3	27	4.5	0.375	12	4.5	0.75	6	4.5	1.5	3	4.5	4.5	1

ESM Table 2: CRS AE Grading System

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Headache	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain: limiting self-care ADL	-	-
Fever	38.0-39.0°C	>39.0-40.0°C	>40.0°C for ≤ 24 hrs	>40.0°C for > 24 hrs	Death
Vomiting	1-2 episodes (separated by 5 minutes) in 24 hrs	3-5 episodes (separated by 5 minutes) in 24 hrs	≥6 episodes (separated by 5 minutes) in 24 hrs	Life-threatening consequences; urgent intervention indicated	Death
Diarrhoea	Increase of <4 stools per day over baseline	Increase of 4-6 stools per day over baseline	Increase of ≥7 stools per day over baseline; incontinence; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated	Death
Hypotension	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Urgent medical intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Chills	Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged; not responsive to narcotics	-	-
Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake	-	-
Arthralgia	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain: limiting self-care ADL	-	-
Myalgia	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain: limiting self-care ADL	-	-

ADL: Activities of daily living

ESM Table 3: Summary of drug-related adverse events by maximum toxicity grade*, n (%)

	Placebo (N=5)	OTX 9 mg (N=9)	OTX18 mg (N=8)	OTX 27 mg (N=7)
Overall				
Any Grade 1 to 5 drug-related event**	5 (100)	9 (100)	8 (100)	7 (100)
Grade 1	3 (60)	1 (11)	3 (38)	2 (29)
Grade 2	2 (40)	4 (44)	2 (25)	3 (43)
Grade 3	0	4 (44)	3 (38)	2 (29)
Dosing period				
Any Grade 1 to 5 drug-related event**	4 (80)	9 (100)	8 (100)	7 (100)
Grade 1	3 (6)	1 (11)	4 (50)	2 (29)
Grade 2	1 (20)	4 (44)	1 (13)	3 (43)
Grade 3	0	4 (44)	3 (38)	2 (29)
Post-dose to week 6				
Any Grade 1 to 5 drug-related event**	3 (60)	7 (78)	7 (88)	7 (100)
Grade 1	2 (40)	7 (78)	6 (75)	4 (57)
Grade 2	1 (20)	0	1 (13)	3 (43)
Grade 3	0	0	0	0
Week 6 to month 24				
Any Grade 1 to 5 drug-related event**	5 (100)	8 (89)	5 (63)	5 (71)
Grade 1	5 (100)	8 (89)	5 (63)	4 (57)
Grade 2	0	0	0	1 (14)
Grade 3	0	0	0	0

*Definition of grades: See ESM Table 2

**There were no Grade 4 or 5 events reported

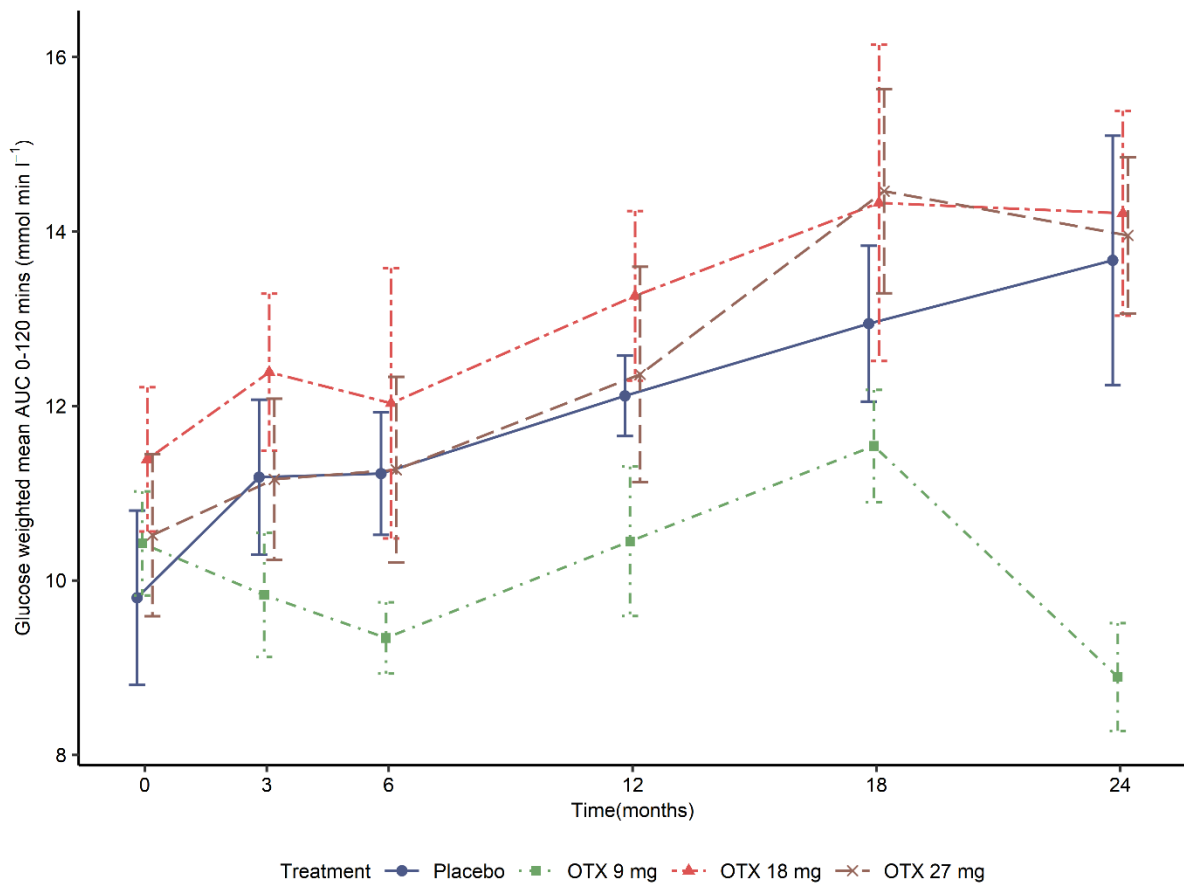
ESM Table 4: Lymphocyte subsets: Mean (Standard Error) change from baseline at month 1/week 6.

Parameter	Placebo	OTX 9 mg	OTX 18 mg	OTX 27 mg
CD3 (10 ⁹ /L) T Lymphocytes	-0.60 (0.408)	0.055 (0.1141)	-0.14 (0.235)	0.98 (0.303)
CD3/Lymphocytes (%) T Lymphocytes	-1.75 (1.109)	-1.33 (1.498)	0.000 (1.2536)	4.67 (1.498)
CD4 (10 ⁶ /L) Th cells	-318 (186.4)	-198 (36.9)	-224 (124.8)	-95.0 (65.36)
CD4/Lymphocytes (%) Th cells	-2.50 (1.708)	-9.33 (1.856)	-5.71 (1.755)	-18.3 (2.29)
CD4+CD25+CD127- (10 ⁶ /L) CD4 Treg	-8.02 (7.696)	-4.19 (5.024)	-23.9 (8.44)	3.44 (5.286)
CD4+CD25+CD127-/CD4+ (%) CD4 Treg	0.39 (0.555)	0.42 (0.949)	-1.29 (0.602)	1.08 (0.798)
CD4+CXCR3+CCR6- (10 ⁶ /L) Th1	-169 (97.1)	-27.2 (43.39)	-48.2 (25.46)	42.4 (28.79)
CD4+CXCR3+CCR6-/CD4+ (%) Th1	-3.26 (6.048)	-1.03 (3.764)	0.62 (3.018)	8.47 (3.232)
CD4+CXCR3-CCR6- (10 ⁶ /L) Th2	-322 (389.9)	-215 (63.8)	-71.0 (157.40)	-76.4 (58.24)
CD4+CXCR3-CCR6-/CD4+ (%) Th2	-9.52 (15.874)	-3.61 (6.437)	4.90 (7.324)	-8.56 (7.949)

Parameter	Placebo	OTX 9 mg	OTX 18 mg	OTX 27 mg
CD4+CXCR3-CCR6+ (10 ⁶ /L) Th17	-37.5 (29.61)	4.65 (27.810)	-33.6 (22.25)	-6.35 (19.133)
CD4+CXCR3-CCR6+/CD4+ (%) Th17	-1.78 (1.541)	1.87 (1.574)	-1.31 (2.148)	0.88 (1.994)
CD8 (10 ⁶ /L) Tc	-105 (81.1)	290 (152.7)	136 (134.5)	1155 (249.3)
CD8/Lymphocytes (%) Tc	1.00 (1.291)	10.0 (3.75)	7.57 (2.359)	24.0 (1.98)
CD8+CD25+CD127- (10 ⁶ /L) CD8 Treg	3.63 (3.790)	0.69 (1.190)	1.35 (0.544)	24.7 (14.01)
CD8+CD25+CD127-/CD8+ (%) CD8 Treg	0.37 (0.429)	-0.050 (0.1053)	0.082 (0.0289)	0.42 (0.394)
CD8+CXCR3+CCR6- (10 ⁶ /L) Tc1	-171 (121.5)	104 (97.5)	187 (66.0)	755 (215.8)
CD8+CXCR3+CCR6-/CD8+ (%) Tc1	-11.8 (9.46)	4.21 (9.394)	11.9 (2.94)	15.5 (5.28)
CD8+CXCR3-CCR6- (10 ⁶ /L) Tc2	-90.4 (117.18)	5.14 (160.167)	-15.9 (94.28)	211 (80.2)
CD8+CXCR3-CCR6-/CD8+ (%) Tc2	-4.54 (9.250)	-6.05 (10.961)	-10.4 (3.44)	-16.0 (5.71)
CD8+CXCR3-CCR6+ (10 ⁶ /L) Tc17	0.94 (1.929)	31.8 (27.88)	-4.93 (2.444)	8.40 (5.533)

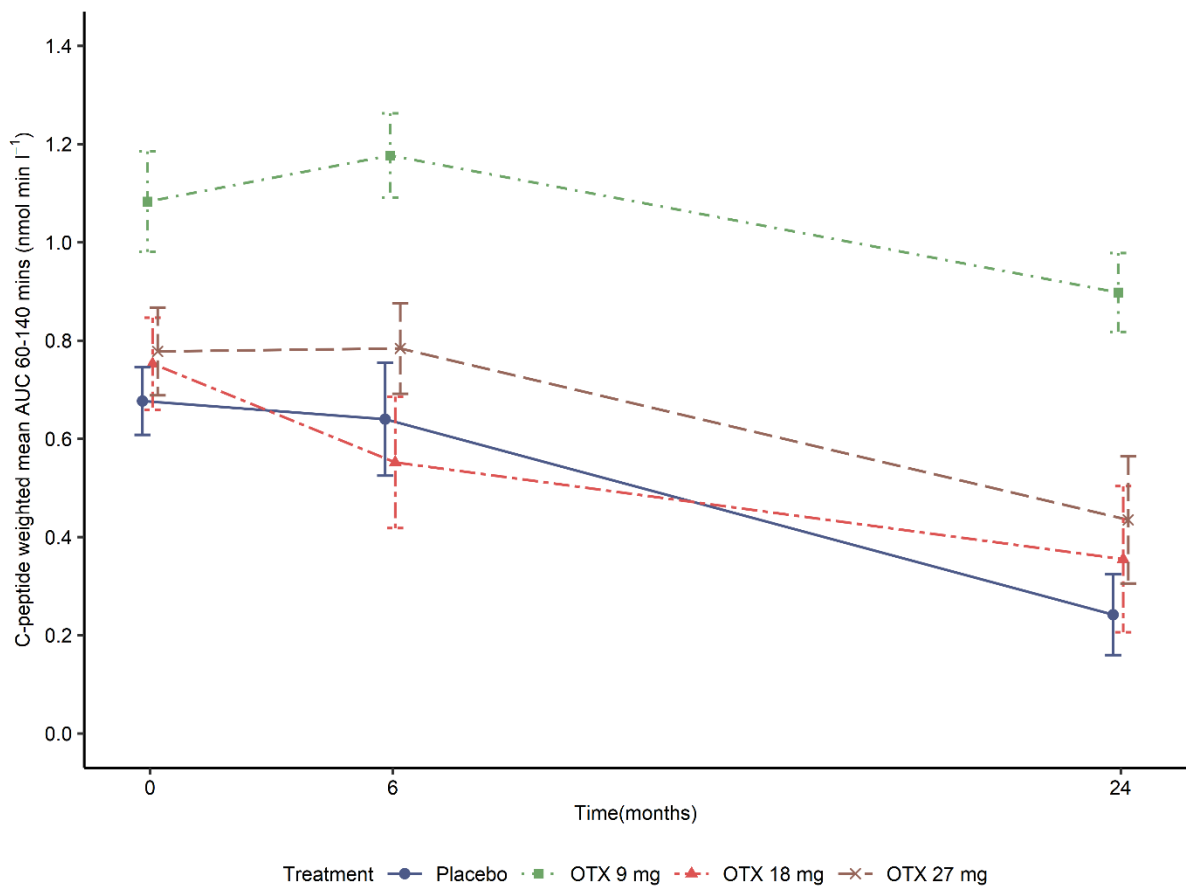
Parameter	Placebo	OTX 9 mg	OTX 18 mg	OTX 27 mg
CD8+CXCR3-CCR6+/CD8+ (%) Tc17	-0.22 (0.547)	2.88 (1.301)	-0.82 (0.309)	-0.63 (0.382)

ESM Figure 1: Glucose weighted mean AUC 0-120 mins post MMTT



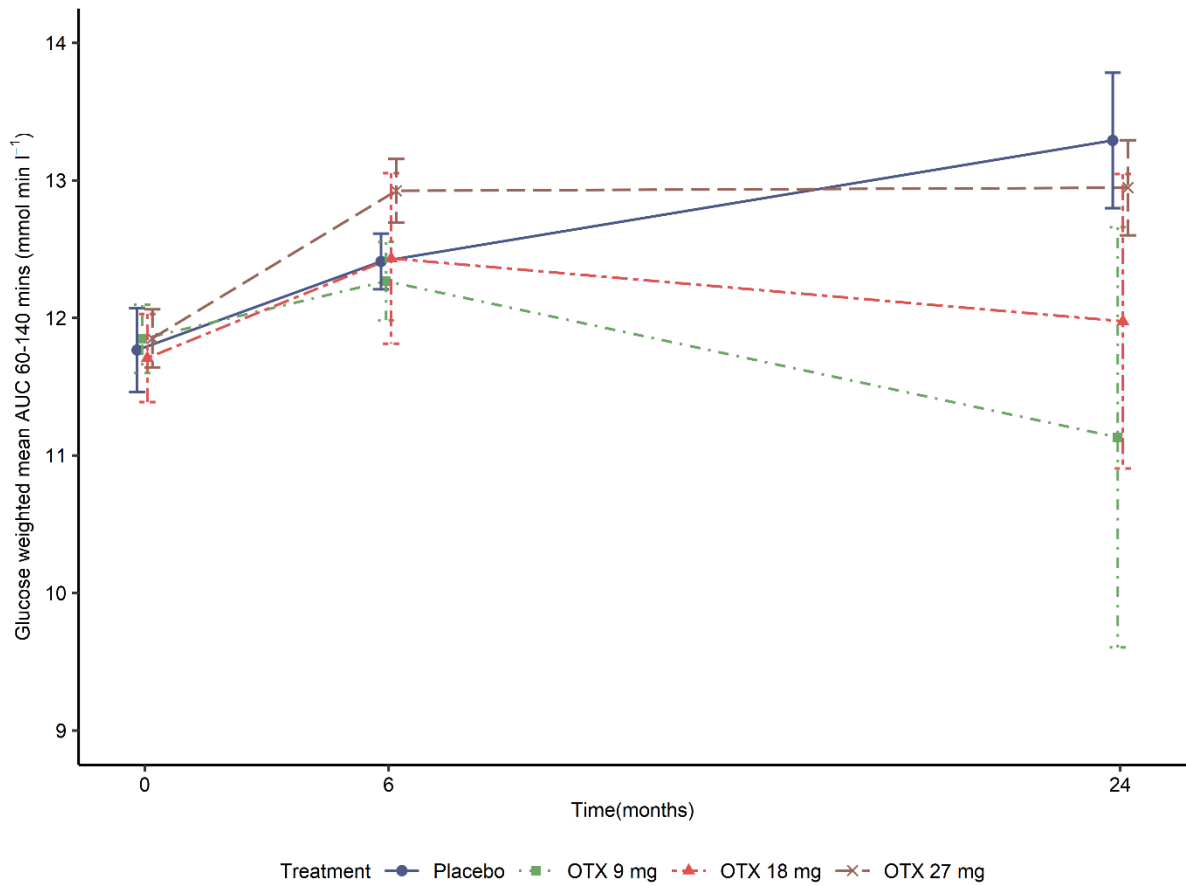
AUC: area under the curve; MMTT: Mixed-meal tolerance test; OTX: Otelixizumab

ESM Figure 2: C-peptide weighted mean AUC 60-140 mins post hyperglycaemic clamp test



AUC: area under the curve; OTX: Otelixizumab

ESM Figure 3: Glucose weighted mean AUC 60-140 mins post hyperglycaemic clamp test



AUC: area under the curve; OTX: Otelixizumab

ESM Figure 4: Example of EBV-reactive CD8+ T-cell frequencies by multimer flow analysis (placebo vs 27 mg)

