Supplementary Appendix

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Inclusion criteria

- Type 1 diabetes
- 18-70 years of age
- Duration of diabetes less than 60 months from diagnosis
- Written informed consent to participate

Exclusion criteria

- Hypersensitivity to aldesleukin or any of the excipients
- History of severe cardiac disease
- History of malignancy within the past 5 years (with the exception of localized carcinoma of the skin that had been resected for cure or cervical carcinoma *in situ*
- History or concurrent use of immunosuppressive agents or steroids.
- History of unstable diabetes with recurrent hypoglycaemia
- Active autoimmune Hyper or hypothyroidism
- History of live vaccination two weeks prior to first treatment
- Active clinical infection
- Major pre-existing organ dysfunction or previous organ allograft
- Females who are pregnant, lactating or intend to get pregnant during the study
- Males who intend to father a pregnancy during the study
- Donation of more than 500 ml of blood within 2 months prior to aldesleukin administration
- Participation in a previous therapeutic clinical trial within 2 months prior to aldesleukin administration
- Abnormal ECG
- Abnormal full blood count, chronic renal failure (Stage 3,4,5) and/or evidence of severely impaired liver function (ALT/AST >3xULN at screening; alkaline phosphatase and bilirubin 2 x ULN at screening (isolated bilirubin >2 x ULN is acceptable if bilirubin is fractionated and direct bilirubin <35%)).
- Positive HBsAg or HepC serology or HIV test
- Any medical history or clinically relevant abnormality that is deemed by the principal investigator/delegate and/or medical monitor to make the patient ineligible for inclusion because of a safety concern.

Study drug administration

The trial drug will be the standard commercial stock of aldesleukin (Marketing Authorisation Holder: Novartis Pharmaceuticals UK Limited). Aldesleukin is produced by recombinant DNA technology using an Escherichia coli strain, which contains a genetically engineered modification of the human interleukin 2 (IL-2) gene.

The drug is provided as powder for solution for injection or infusion. For all injections, aldesleukin is first reconstituted under aseptic conditions as per Proleukin® SmPC. Solutions are then further diluted to the required concentration in glucose 5% and the appropriate volume drawn up into sterile U-100 insulin syringes.

The first dose of aldesleukin will be self-administered by participants at the trial site following instruction by an appropriately trained delegated member of the trial team. Participants can then administer further doses either at the trial site or in the community. Alternatively, doses may be administered by an appropriately qualified member of the trial team. The date and time of each dose administered at the trial site will be recorded in the source documents. In the community, the date and time of each dose administered will be recorded in the participant's study diary that will become part of the source documents. The study diary, injection sites and count of unused medication will be carried out at each study visit while on treatment to confirm compliance.

Drug storage and supply

Commercial supply of aldesleukin, obtained via usual hospital route, will be used for this study. Vials will be stored as per SmPC. at 2ºC to 8ºC (in a refrigerator) in the original package in order to protect from light. The vials must not be frozen. Vials will be stored securely with access restricted to appropriate individuals.

Previous (unlicensed) data supports stability and sterility of reconstituted diluted IL-2 preparations (reconstituted with Water for Injection as per the Proleukin® SmPC and further diluted with glucose 5%) for up to 14 days at 2-8ºC when syringes are prepared by qualified health-care professionals under aseptic conditions. A maximum shelf life of 7 days will be assigned to the prepared doses for this study.

The pharmacy at the participating site will dispense aldesleukin for this trial upon receipt of a suitably signed trial specific prescription. For each individual participant, multiple individual doses of a single concentration of the aldesleukin may be prepared under aseptic conditions for administration in individual insulin syringes for each 7 days of the treatment period. All doses not used within the 7-day period will be returned to the pharmacy at participating site and a record of their disposal will be made.

Co-primary endpoints - Definitions of Tregs, Teffs and CD25 expression within the $CD3^+CD4^+$ fluorescence-activated cell sorting (FACS) gate

Tregs: Tregs % $CD25^{high}CD127^{low}$ within the $CD3^+CD4^+$ gate

CD25 expression on Tregs: Mean fluorescence intensity (MFI) of CD25 allophycocyanin (APC) within the Treg ($CD3^+CD4^+CD25^{high}CD127^{low}$) gate

Teffs: Teff populations (non-Tregs) account for all the other $CD3^+CD4^+$ cells that are not defined as Tregs within the $CD3^+CD4^+$ gate:

- Effector memory % $CD45RA^-CD62L^-$
- Central memory % $CD45RA^-CD62L^+$
- Naïve Teffs % $CD45RA^+CD62L^+$
- Effector memory RA+ (TEMRA) $CD45RA^+CD62L^-$
- Central memory %+ Effector memory % = Total Memory effectors %
- Change in the ratio Naïve effectors %: Total Memory effectors % defines the Teff primary endpoint.

DOSE AND FREQUENCY COMMITTEE CHARTER: DILFREQUENCY TRIAL

SCOPE

To provide decisions regarding the choice of dose and frequency of aldesleukin to administer to participants in the confirming phase (groups 5,6 &7)

INPUT DATA

After each group has completed their dosing schedule the data with be extracted from the DILfrequency OpenClinica database. The data will be delivered to the Dose and Frequency Committee (DFC) within 2 working days of that date.

The data will include:

- Assessments of safety
- Dose and frequencies assigned for all participants in preceding groups.
- All CD4 Tregs, CD4 effectors and CD25 expression on Tregs data available from visits

DECISIONS

- Determine if steady state has been achieved
- Define the visit numbers for measurement of the three trough values for each participant
- Define the numerical targets for Treg increase, increased CD25 expression and minimal increase in Teffs
- Determine if further learning phase is needed
- Define the dose and frequency for the next cohort of participants
- Safety assessment – Clinician only

TIMELINES

- At the first meeting a DFC report prepared by the statisticians will be circulated to members of the committee.
- The first meeting of the DFC will be scheduled within 21 days of the last participant of the learning phase completing the dose schedule
- A second meeting of the DFC will be scheduled within 14 days of the first meeting of the DFC
- The third meeting of the DFC will be scheduled within 28 days of the first meeting of the DFC.
- Further meetings of the DFC will be scheduled within 14 days of each group completing their dosing schedule a report will be issued to committee members 7 days in advance.

DFC MEETINGS

- Statisticians will receive a data download from OpenClinica of the Input data and will prepare a DFC report.

Learning phase

- The first DFC meeting will occur no less than 7 days after the DFC report of the learning phase has been circulated to members and within 21 days of the learning phase completion.

    - The purpose of the first meeting will be to discuss whether steady state has been achieved in the learning phase, define the visit numbers for the three trough values, to review safety data, review the report formatt and to discuss targets.
- The second DFC meeting will occur within 14 days of the first meeting. A second DFC revised report in light of the comments from the first meeting will be prepared and distributed to committee members 1 week before meeting 2
    - The purpose of the meeting will be to decide the targets for the remainder of the trial if adequate input data has been acquired in the trial at this stage.
    - In the event that the inadequate input data is available the DFC may decide on a further learning phase.
- The third DFC meeting will occur within 28 days of the first meeting. A third DFC report will be prepared and distributed to committee members 1 week before meeting 3
    - The purpose of the meeting will be allocate the dose(s) and frequency(s) to administer to the next cohort of participants

Confirming phase

- Subsequent meetings will occur following completion of dosing schedules for each participant cohort and will decide the dose(s) and frequency(s) to administer to the next cohort of participant.

Meetings will be organised by the Trial Coordinator or delegate on behalf of the CI and will be in person ideally or by teleconference or email.

Reporting

The report prepared from the data will include

- Plots of all the patient profiles (Treg, CD25, Teff response versus time)
- Plots of the sequence of doses and dose intervals
- Scatter plots of the co-primary endpoints (average of the trough values) versus dose and dose interval.
- Scatter plots of the co-primary endpoint adjusted for covariates versus dose and dose-interval with superimposed fitted linear regression models with 95% confidence bands reporting
    - Log-likelihood
    - Raw output from statistical packages to double-check on convergence
    - Residual plots of each model fitted.

The details of the report may evolve as the trial progresses.

Minutes

Minutes will be produced by the chair or delegate and stored in the Trial Master File. Responsibility for communicating any decisions to the study team lies with the chair.

Membership

The roles may be taken from the following groups of staff and more than one member from each role may attend

- Clinicians - Frank Waldron-Lynch, Mark Evans, David Dunger, Sankalpa Neupane, Lucy Truman, Ben Challis
- Statisticians - Adrian Mander, Simon Bond

- Scientists - Linda Wicker, John Todd, Linsey Porter

The Chair is Dr. Frank Waldron-Lynch or if absent an alternative agreed by the committee

A member may temporarily delegate their responsibilities to another suitably qualified person if agreed within the committee. Observers may attend if this is agreed by the committee. Permanent changes of membership need to be approved by the TSC.

QUORUM AND VOTING

A minimum of a statistician, a clinician, and a scientist are required to be quorate. Each role has a single vote and decisions can be reached by a majority opinion. The TSC may be called upon to resolve any decisions that cannot be agreed upon.

Chief Investigator

Name: Frank Waldron-Lynch

Signature: FWL-1.

Date: 9/1/2015

Supplementary tables

Table S1 Dose and frequency allocation according to cohort

LEARNING	**COHORT 1**				
		0.09 x10^6 IU/m^2	*0.47 x10^6 IU/m^2*	*0.20 x10^6 IU/m^2*	*0.32 x10^6 IU/m^2*
	14 daily	2	2		
	10 daily		2		
	5 daily	2			
	2 daily	2	2		
	COHORT 2				
	10 daily	1			
	5 daily		2		
	4 daily	1	1		
	3 daily	1	2		
CONFIRMING	**COHORT 3**				
	3 daily			5	4
	COHORT 4				
	3 daily				8

Table S2 Dose and frequency allocation according to population.

SAFETY POPULATION				
	0.09 x10^6 IU/m^2	*0.47 x10^6 IU/m^2*	*0.20 x10^6 IU/m^2*	*0.32 x10^6 IU/m^2*
14 daily	2	2	0	0
10 daily	1	2	0	0
5 daily	2	2	0	0
4 daily	1	1	0	0
3 daily	1	2	5	12
2 daily	2	2	0	0
ANALYSIS POPULATION				
14 daily	1	2	0	0
10 daily	1	2	0	0
5 daily	2	2	0	0
4 daily	1	0	0	0
3 daily	1	2	4	8
2 daily	1	1	0	0

Table S3. Adverse events by severity and type in the safety population (N= 37)

Type of Adverse event	Category	Events	Participants
Total AEs	Most common AEs (>10%)		
	- Hypoglycaemia	94 (13.5%)	20 (54.1%)
	- Injection site erythema	230 (33.1%)	35 (94.6%)
	- Injection site nodule	234 (33.7%)	33 (89.2%)
	Adverse events		
	- Unexpected	127 (18.3%)	34 (91.9%)
	- Expected adverse events	567 (81.7%)	37 (100%)
	- Related	479 (69.1)	
	- Unrelated	215 (31.0%)	
Unexpected	Unexpected adverse events by severity (N/%)		
	- Mild	108 (85%)	32 (86.5%)
	- Moderate	19 (15%)	11 (29.7%)
	Most common (>10%) unexpected AEs		
	- Headache	22 (17.3%)	12 (32.4%)
	- Nasopahryngitis	18 (14.2%)	16 (43.2%)
	Unexpected adverse events (N/%) by relatedness		
	- Unrelated	120 (94.5%)	
	- Related	7 (5.5%)	
Expected	Expected adverse events by severity (N/%)		
	- Mild	562 (99.1%)	37 (100%)
	- Moderate	5 (0.9%)	3 (8.1%)
	Most common expected AEs (>10% AEs)		
	- Hypoglycaemia	94 (16.6%)	20 (54.1%)
	- Injection site erythema	230 (40.6%)	35 (94.6%)
	- Injection site nodule	234 (41.3%)	33 (89.2%)
	Expected AEs		
	- Related	472 (83.2%)	
	- Unrelated	95 (16.8%)	
Unrelated AEs	Unrelated adverse events by severity (N/%)		
	- Mild	196 (91.2%)	35 (94.59%)
	- Moderate	19 (8.8%)	11 (29.73%)
	Most common unrelated AEs (>10%)		
	- Headache	22 (10.2%)	12 (32.4%)
	- Hypoglycaemia	94 (43.7%)	20 (54.1%)
	Unrelated adverse events (N/%)		
	- Expected	95 (44.2%)	21 (56.7%)
	- Unexpected	120 (55.8%)	34 (91.9%)
Related AEs	Drug-related AEs according to cohorts		
	Cohort 1		
	- Injection site erythema		
	- Injection site nodule	55 (49.1%)	11 (91.7%)
	- Nausea and vomiting symptoms	56 (50.0%)	10 (83.3%)
	Cohort 2	1 (0.9%)	1 (8.3%)
	- Acne		
	- Eosinophilia	2 (2.0%)	2 (25.0%)
	- Injection site erythema	1 (1.0%)	1 (12.5%)
	- Injection site nodule	50 (49.0%)	7 (87.5%)

	- Malaise	47 (46.1%)	6 (75.0%)
	- Migraine headaches	1 (1.0%)	1 (12.5%)
	Cohort 3	1 (1.0%)	1 (12.5%)
	- Injection site erythema		
	- Injection site nodule	68 (55.3%)	9 (100%)
	- Oropharyngeal pain	54 (43.9%)	9 (100%)
	Cohort 4	1 (0.8%)	1 (11.1%)
	- Asthenic condition		
	- Dry eye	3 (2.1%)	3 (37.5%)
	- Injection site erythema	1 (0.7%)	1 (12.5%)
	- Injection site nodule	57 (40.1%)	8 (100%)
	- Nasopharyngitis	77 (54.2%)	8 (100%)
	Nausea and vomiting symptoms	3 (2.1%)	3 (37.5%)
		1 (0.7%)	1 (12.5%)
	Drug related injection site reactions per number of doses		
	- Injection site erythema per number of dose	230/323 (71.2%)	
	- Injection site nodule per number of dose	234/323 (72.4%)	
	Drug related injection site reactions per number of doses per cohort		
	Cohort 1		
	- Injection site erythema per number of dose	55/78 (70.5%)	11 (91.7%)
	- Injection site nodule per number of dose	56/78 (71.8%)	10 (83.3%)
	Cohort 2		
	- Injection site erythema per number of dose	50/80 (62.5%)	7 (87.5%)
	- Injection site nodule per number of dose	47/80 (58.8%)	6 (75.0%)
	Cohort 3		
	- Injection site erythema per number of dose	68/85 (80.0%)	9 (100%)
	- Injection site nodule per number of dose	54/85 (63.5%)	9 (100%)
	Cohort 4		
	- Injection site erythema per number of dose	57/80 (71.3%)	8 (100%)
	- Injection site nodule per number of dose	77/80 (96.3%)	8 (100%)

Table S4. Safety parameters (safety population)

Parameter	**Baseline**	**Follow up**	**Normal range**
hsCRP (mg/L)	2.55 (±5.72)	1.90 (±2.47)	≤3
Sodium (mmol/L)	138.75 (±2.32)	138.6 (±2.4)	133-146
Potassium (mmol/L)	4.36 (±0.25)	4.35 (±0.28)	3.5-5.3
Albumin corrected calcium (mmol/L)	2.32 (±0.09)	2.36 (0.13±)	2.08-2.65
Urea (mmol/L)	5.55 (±1.6)	5.69 (±1.34)	2.5-7.8
Creatinine (umol/L)	72.39 (±14.92)	71.3 (±11.3)	F: 44-97 M: 62-115
Alanine transaminase (U/L)	21.97 (±15.42)	24.9 (±22.7)	7-40
Aspartate aminotransferase (U/L)	20.31(±7.23)	22.71 (±12.76)	≤ 34
GGT (U/l)	17.11 (±12.04)	20.9 (±23.8)	F: 0-37 M: 0-72
Alkaline phosphatase (U/L)	74.11 (±20.25)	80.8 (±23.9)	30-130
TSH (mU/L)	1.55 (±0.92)	1.85 (±1.06)	0.35-5.50
Free T4 (pmol/L)	15.42 (±2.4)	15.06 (±1.74)	10.0-19.8
Red blood cells (10^9/l)	4.79 (±0.42)	4.74 (±0.40)	F: 3.90-5.20 M: 4.00-5.65
Haemoglobin	144.9 (±12.2)	142.0 (±13.3)	F: 120-156 M: 135-172
Haematocrit (L/L)	0.42 (±0.03)	0.41(±0.03)	F: 0.355-0.455 M: 0.395-0.505
Mean cell haemoglobin (pg)	30.21 (±1.53)	29.93 (±1.8)	F: 27.0-33.5 M: 27.0-33.5
Red cell distribution width (%)	13.2 (±0.54)	13.05 (±0.71)	11.0-16.0
Platelet count (10^9/l)	224.4 (±44.4)	234.5 (±51.6)	150-370
White blood cells (10^9/l)	5.71 (±1.76)	5.57 (±1.65)	3.90-10.20
Neutrophils (10^9/l)	3.49 (±1.61)	3.36 (±1.36)	1.50-7.70
Lymphocytes (10^9/l)	1.53 (±0.37)	1.56 (±0.34)	1.50-7.70

Monocytes (10^9/l)	0.41 (±0.14)	0.42 (±0.16)	0.10-0.90
Eosinophils (10^9/l)	0.16 (±0.16)	0.15 (±0.10)	0.10-0.90
Basophils (10^9/l)	0.05 (±0.04)	0.04 (±0.02)	0.00-0.20

Table S5. Clinical FACS analysis (safety population)

Parameter	**Baseline**	**Follow up**
CD19%	12.2 (±3.9)	12.3 (±4.3)
CD19 Total B cells (*10^9 /L)	0.2168 (±0.17)	0.19 (±0.08)
CD3%	74.8 (±6.1)	75.1 (±5.6)
CD3 Total T cells (*10^9 /L)	1.16 (±0.30)	1.19 (±0.27)
CD4%	47.1 (±7.5)	48.1 (±8.1)
CD4 Total (*10^9 /L)	0.73 (±0.23)	0.76 (±0.21)
CD56%	12.4 (±5.8)	12.1 (±5.6)
CD56 (*10^9 /L)	0.19 (±0.09)	0.18 (±0.08)
CD8%	25.4 (±6.6)	24.9 (±6.7)
CD8 Total (*10^9 /L)	0.39 (±0.13)	0.39 (±0.14)
Lymphocytes count (*10^9 /L)	1.53 (±0.37)	1.58 (±0.33)
Treg % CD4 T cells	7.13 (±1.66)	7.28 (±1.87)
Treg Absoulte (*10^9 /L)	0.05 (±0.02)	0.05 (±0.02)
Treg CD25 Mean FI	2401.8 (±794.7)	2292.4 (±748.1)
Treg CD25 MEF	2863.8 (±754.9)	2558.7 (±520.5)
Non-Treg gated panel		
Naïve %	42.4 (±15.1)	39.6 (±15.3)
Naïve absolute (*10^9 /L)	0.28 (±0.14)	0.28 (±0.15)
Temra %	1.01 (±1.95)	0.7 (±1.7)
Temra absolute (*10^9 /L)	0.006 (±0.013)	0.004 (±0.01)
Total memory %	56.6 (±15.9)	59.9 (±15.9)
Total memory absolute (*10^9 /L)	0.38 (±0.16)	0.41 (±0.14)
Naïve/ memory ratio	0.94 (±0.84)	0.84 (±0.79)

Table S6. AIC for all models (analysis population)

Model	**Treg AIC**	**CD25 AIC**	**Teff AIC**
Linear dose, linear ferquency	245.8	235.93	243.89
Quadratic dose, linear frequency	243.55	236.14	245.85
Linear dose, quadratic frequency	245.72	219.52	240.79
Quadratic dose, quadratic frequency	245.27	215.0	238.63
Log dose, linear frequency	244.26	235.04	243.95
Linear dose, log frequency	244.27	228.82	244.73
Log dose, log frequency	242.88	228.29	244.71
Linear dose, quadratic frequency, interaction	246.66	213.44	236.82

Table S7. Estimated target dose/ frequency

Model	**Best dose**	**Dose SE**	**Dose CI**	**Best Frequency**	**Frequency SE**	**Frequency CI**
Linear dose, linear frequency	-0.082	1.927	-3.858, 3.694	-2.9	31.9	65.4, 59.6
Quadratic dose, linear frequency	0.133	0.154	-0.17, 0.435	-0.7	10.1	-20.4, 19.0
Linear dose, quadratic frequency	0.240	0.114	0.017, 0.463	3.0	0.5	1.7, 3.5
Quadratic dose, quadratic frequency	0.186	0.048	0.093, 0.279	2.6	0.5	1.7, 3.5
Log dose, linear frequency	0.067	0.420	-0, 757, 0.891	-2.5	27.4	-56.3, 51.2
Linear dose, log frequency	0.170	0.482	-0.775, 1.115	2.4	2.7	-2.8, 7.7
Log dose, log frequency	0.168	0.252	-0.327, 0.662	2.5	2.2	-1.8, 6.9
Linear dose, quadratic frequency, interaction	0.239	0.126	-0,008, 0.485	2.9	0.8	1.4, 4.4

Table S8. Best estimated allowable does/ frequency according to Mahalanobis distance and joint probability – Linear dose, quadratic frequency, interaction (analysis population)

Dose	**Frequency**	**Mahalanobis Distance**	**Joint Probability**
0.26	3	0.044	0.742
0.25	3	0.062	0.737
0.27	3	0.111	0.716
0.24	3	0.134	0.702
0.28	3	0.186	0.661

Table S9. Best estimated allowable dose/ frequency according to Mahalanobis distance and joint probability - Linear dose, quadratic frequency, interaction (evaluable population)

Dose	**Frequency**	**Mahalanobis Distance**	**Joint Probability**
0.21	3	0.124	0.696
0.22	3	0.12	0.694
0.2	3	0.16	0.663
0.23	3	0.151	0.658
0.19	3	0.213	0.603

Supplementary Figures

Figure S1. Eosinophilia for individual participants (safety population)

One participant on aldesleukin 0.47 x 10^6 IU/m^2 every 4 days developed transient eosinophilia (red); pre-existing eosinophilia resolved in two participants receiving 0.32 x 10^6 IU/m^2 every 3 days (purple), and 0.09 x 10^6 IU/m^2 every 2 days (blue), respectively.

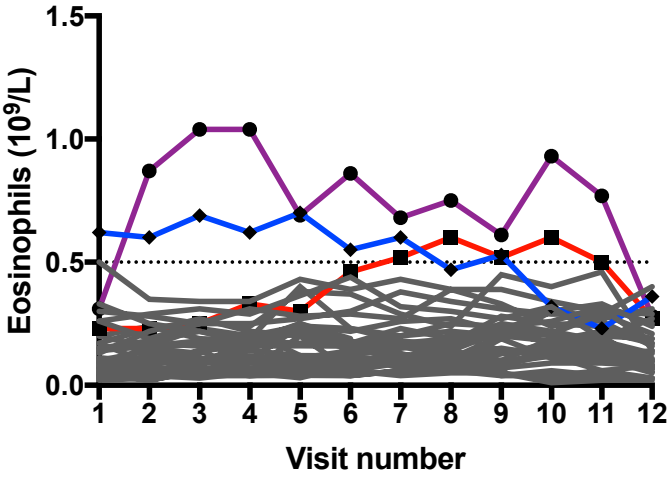


Figure S2. TPO antibodies and TSH receptor antibodies. Data shown for individual patients in safety population.

No participant developed de novo TPO antibodies (A) or TSH receptor antibodies (B) during the trial. Ticked line indicates upper limit of normal range.

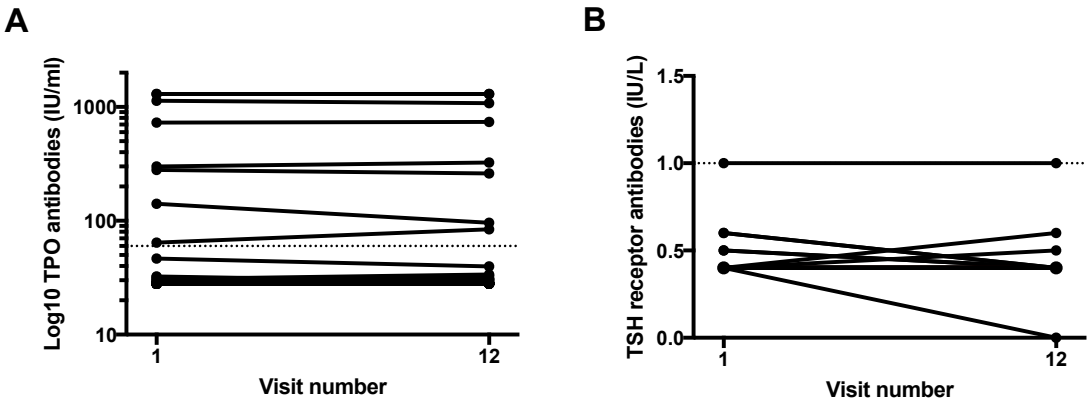


Figure S3. Clinical images and immunohistochemical staining of psoriasis in one participant receiving treatment with aldesleukin 0.32x10^6 IU/m^2 every three days.

(A-F) Clinical images: There was no change in plaque size, and plaque number (three lesions on right elbow, abdomen, and left ear; data not shown) during treatment. Image A to E show plaque on elbow at subsequent dosing visits 3 (A), 4 (B), 8 (C), 9 (D), and 10 (E). At follow up, an additional plaque was observed on the abdomen, while the size of pre-existing plaques remained stable (F).

(G-L) Immunohistochemical staining: skin punch biopsies of the psoriatic plaque on right elbow were taken at visit 10 and at follow up visit 12. Histopathology sections were stained for CD3 before (G) and after (J) treatment, CD4 before (H) and after (K) treatment, and FOXP3 before (I) and after (L) treatment. Images are shown 10x enlarged. CD3, CD4 and FOXP3 positive cells were counted in five random fields of view at 100x magnification. FOXP3 to CD3 ratio (0.17 before, 0.17 after) and FOXP3 to CD4 ratio (0.13 before, 0.15 after) remained unchanged.

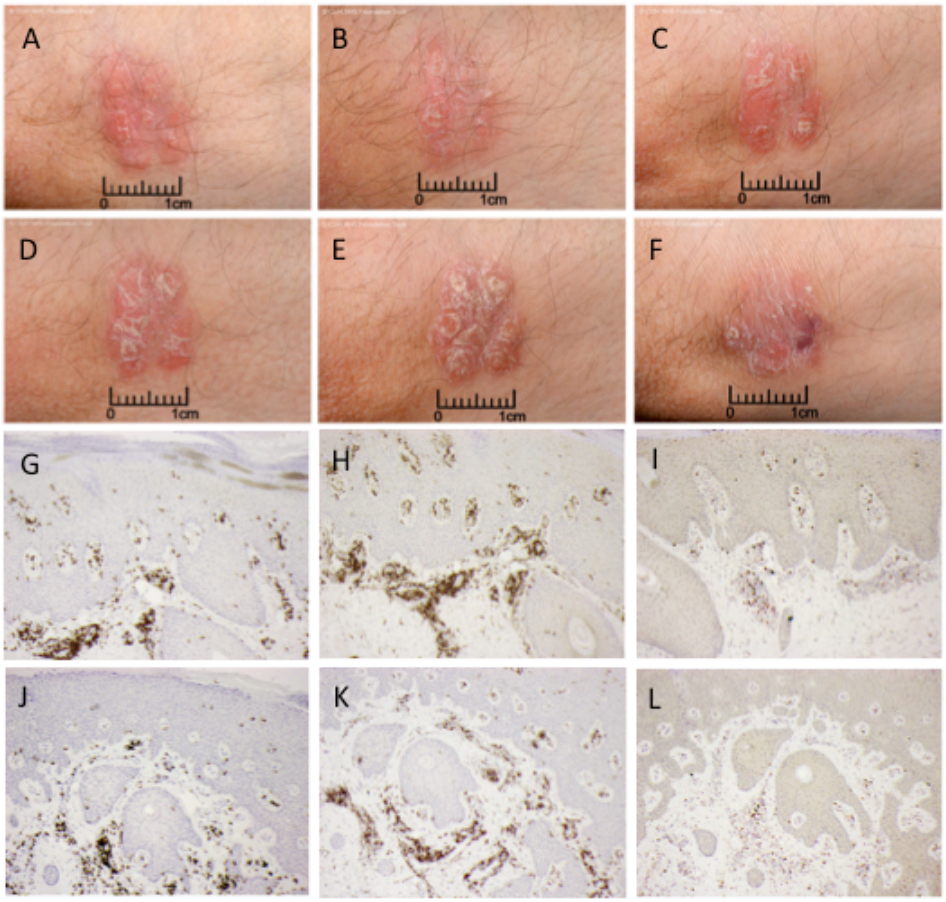


Figure S4: Percentage change of CD19 % in individual participants (each line) by dose and frequency (safety population)

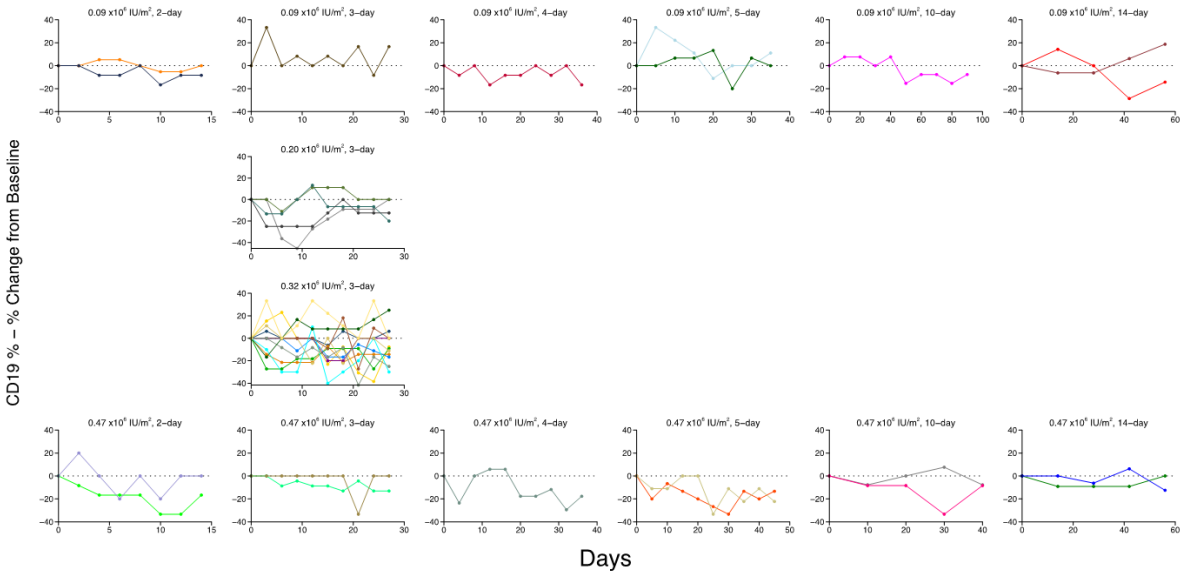


Figure S5: Percentage change of CD19 total B cells in individual participants (each line) by dose and frequency (safety population)

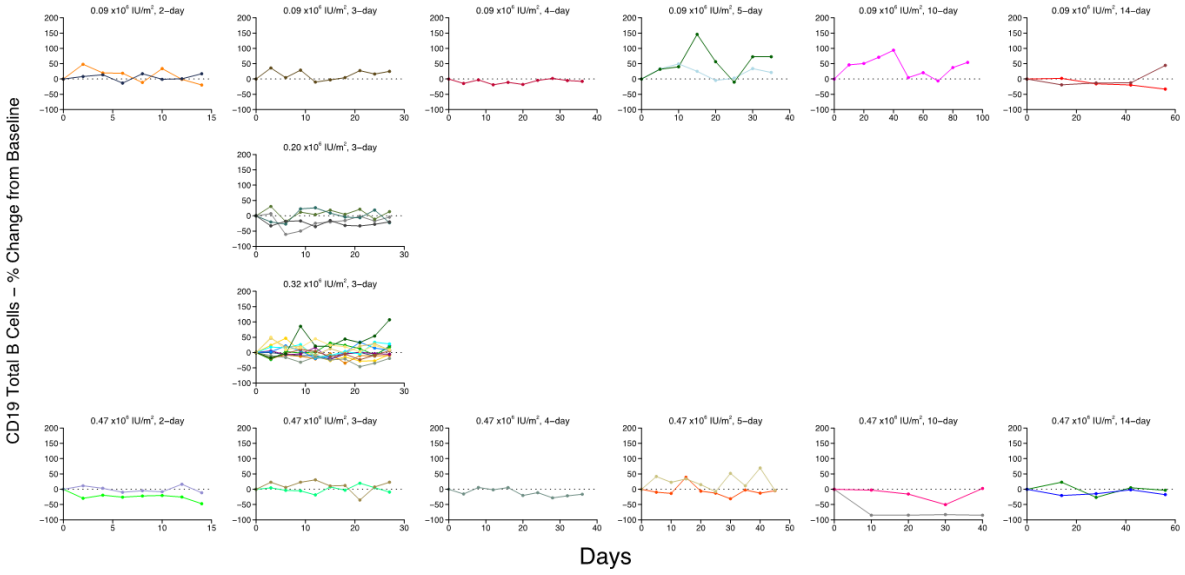


Figure S6: Percentage change of CD3 % in individual participants (each line) by dose and frequency (safety population)

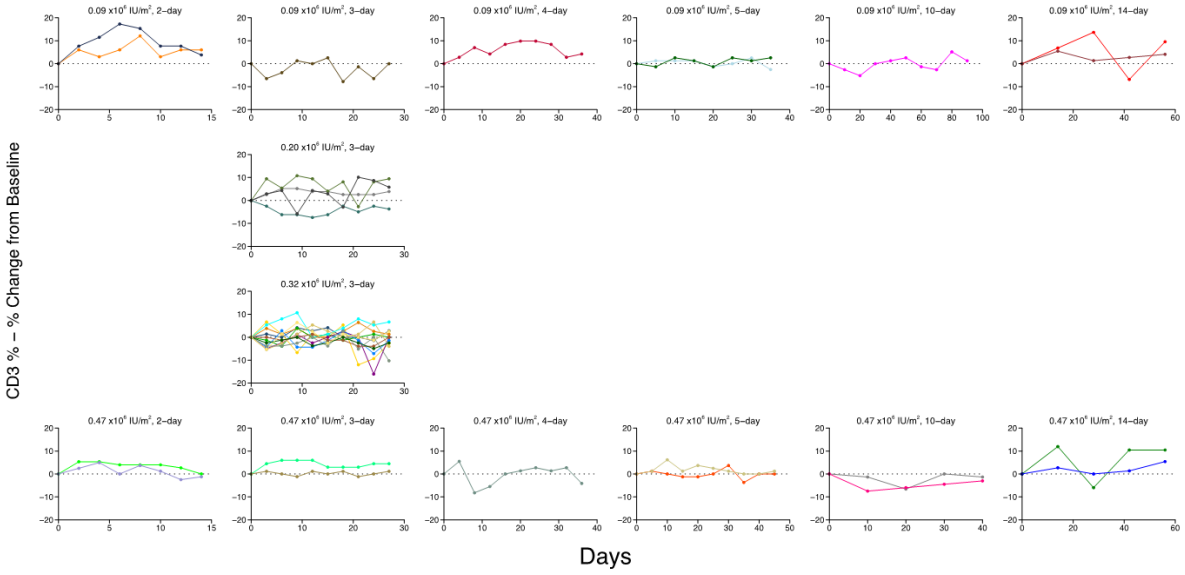


Figure S7: Percentage change of CD3 total T cells in individual participants (each line) by dose and frequency (safety population)

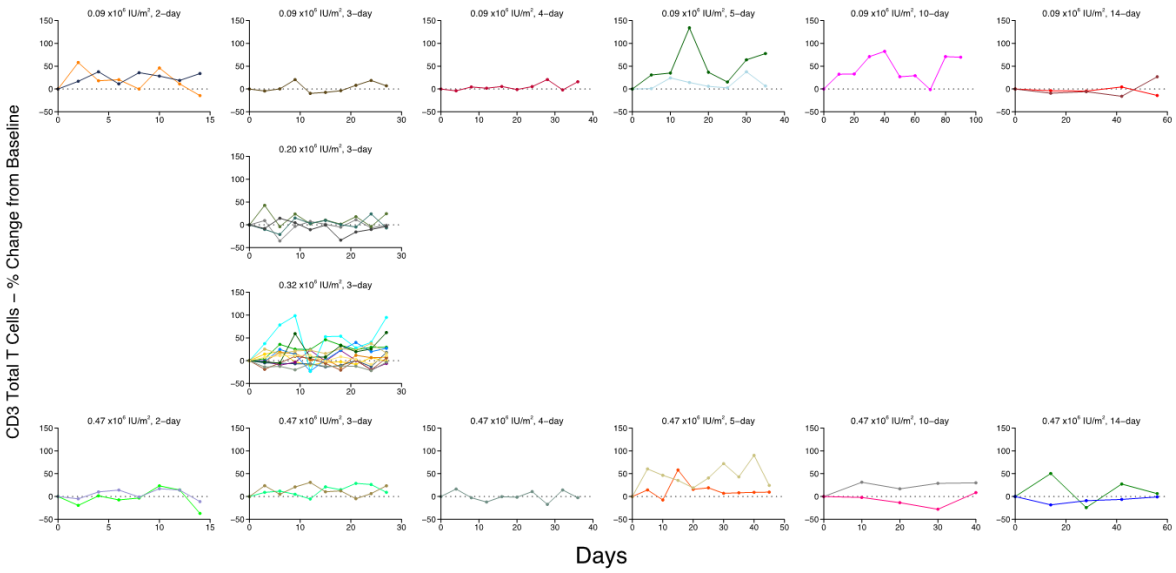


Figure S8: Percentage change of CD4 % in individual participants (each line) by dose and frequency (safety population)

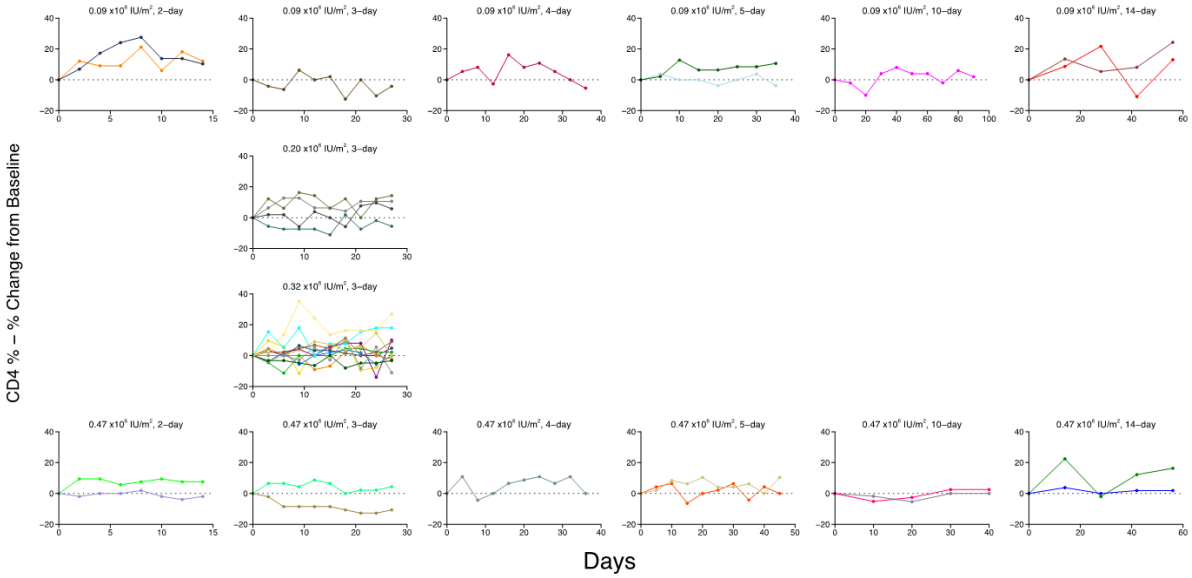


Figure S9: Percentage change of CD4 total in individual participants (each line) by dose and frequency (safety population)

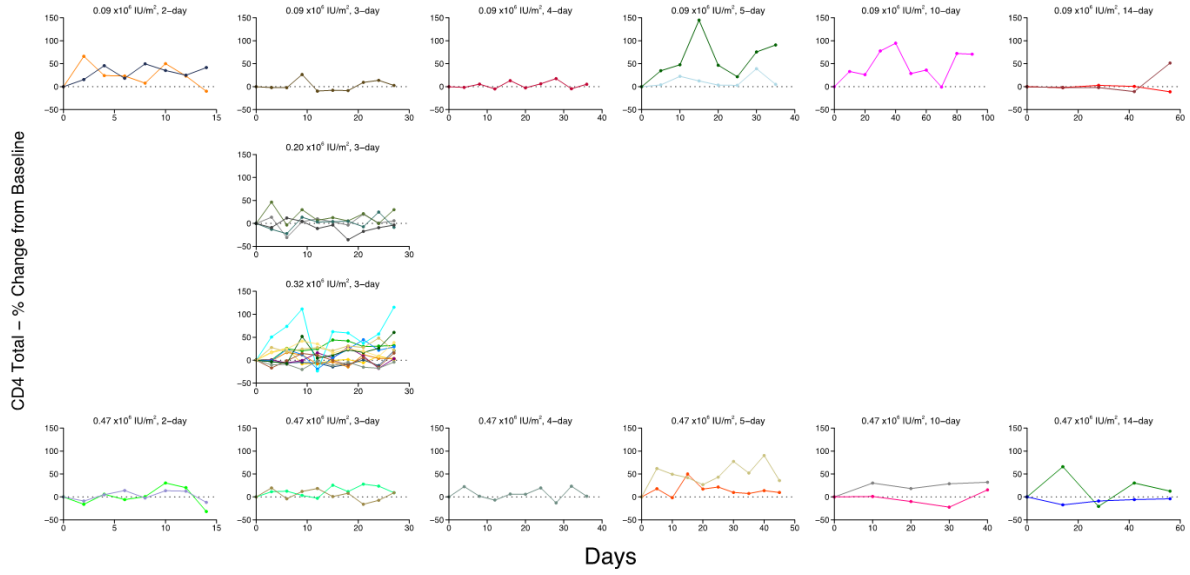


Figure S10: Percentage change of CD56 % in individual participants (each line) by dose and frequency (safety population)

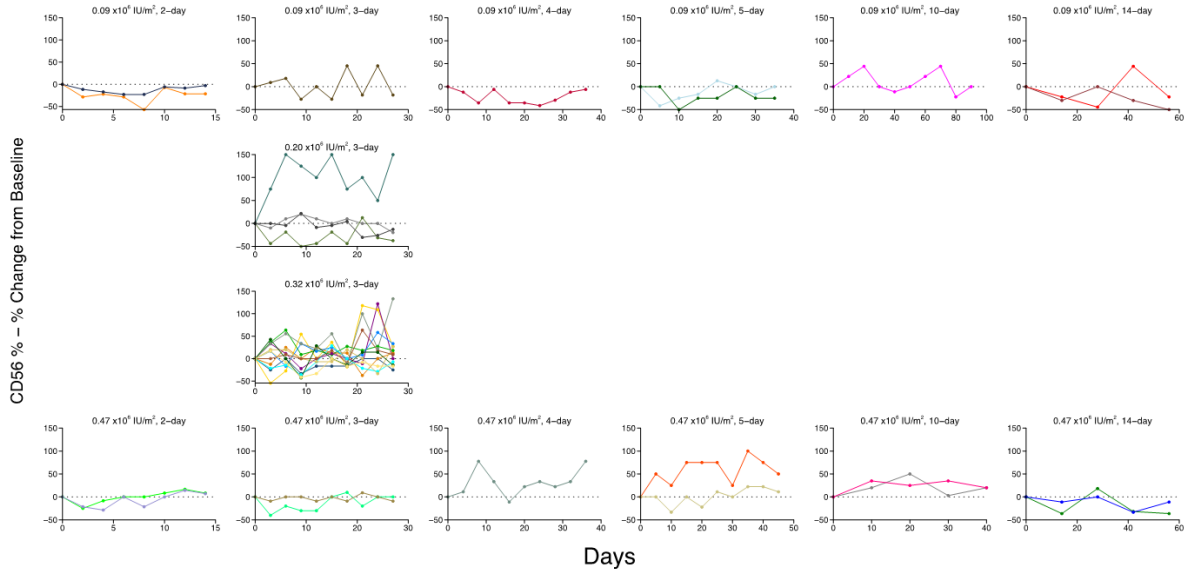


Figure S11: Percentage change of CD56 total NK cells in individual participants (each line) by dose and frequency (safety population)

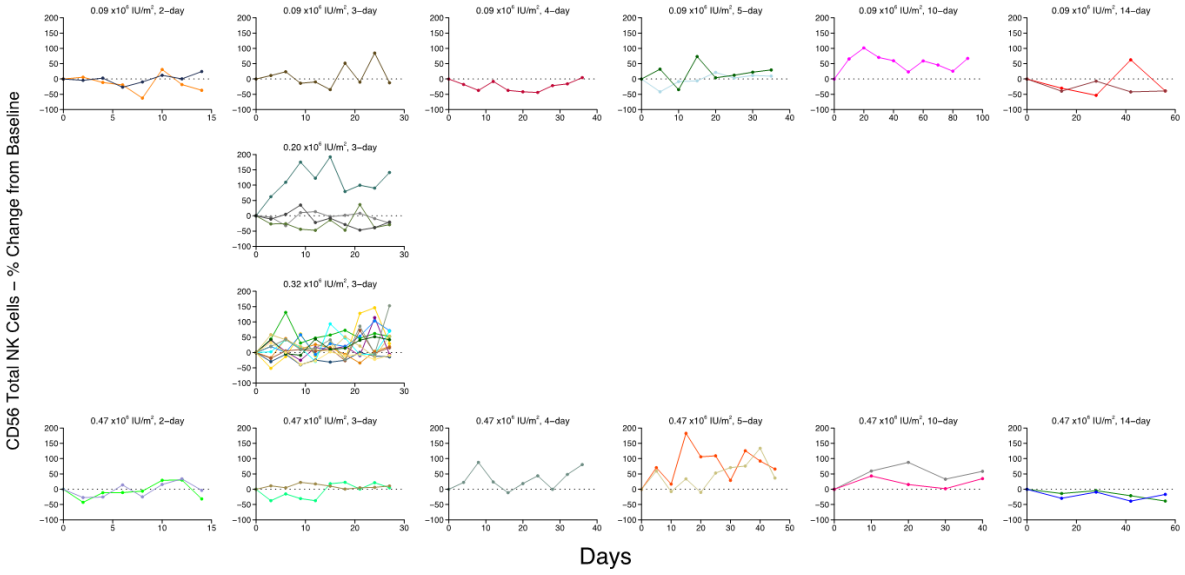


Figure S12: Percentage change of CD8 % in individual participants (each line) by dose and frequency (safety population)

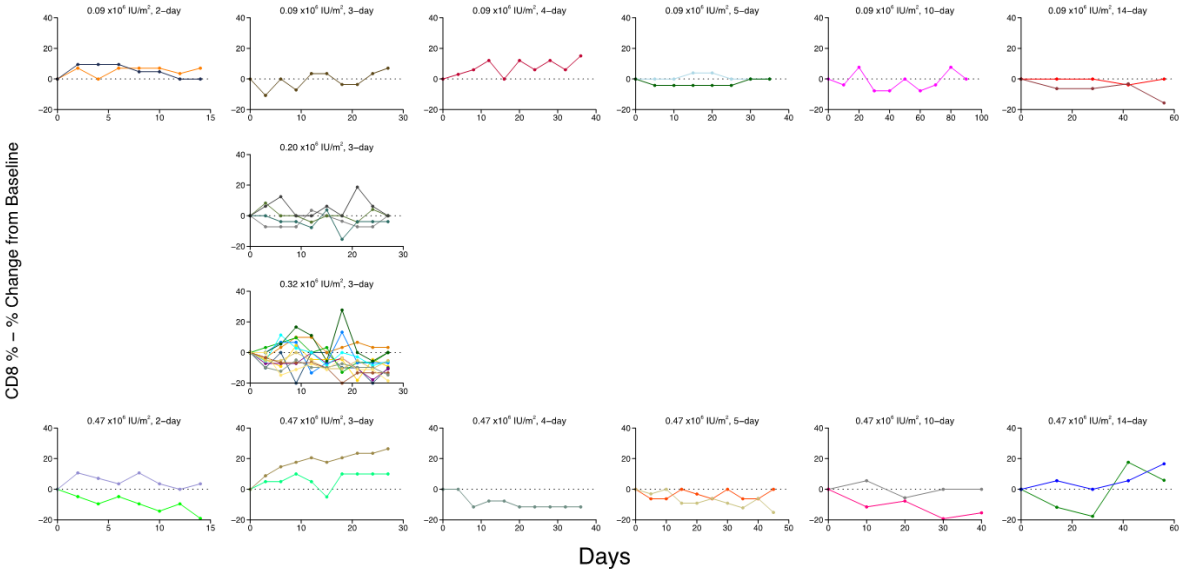


Figure S13: Percentage change of CD8 total in individual participants (each line) by dose and frequency (safety population)

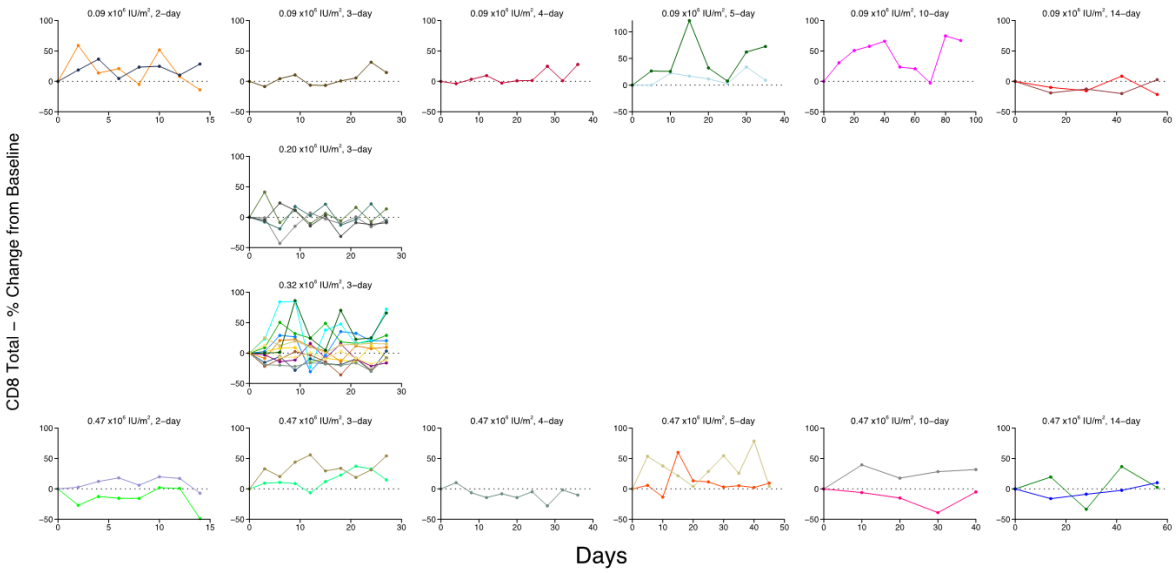


Figure S14: Percentage change of lymphocytes count in individual participants (each line) by dose and frequency (safety population)

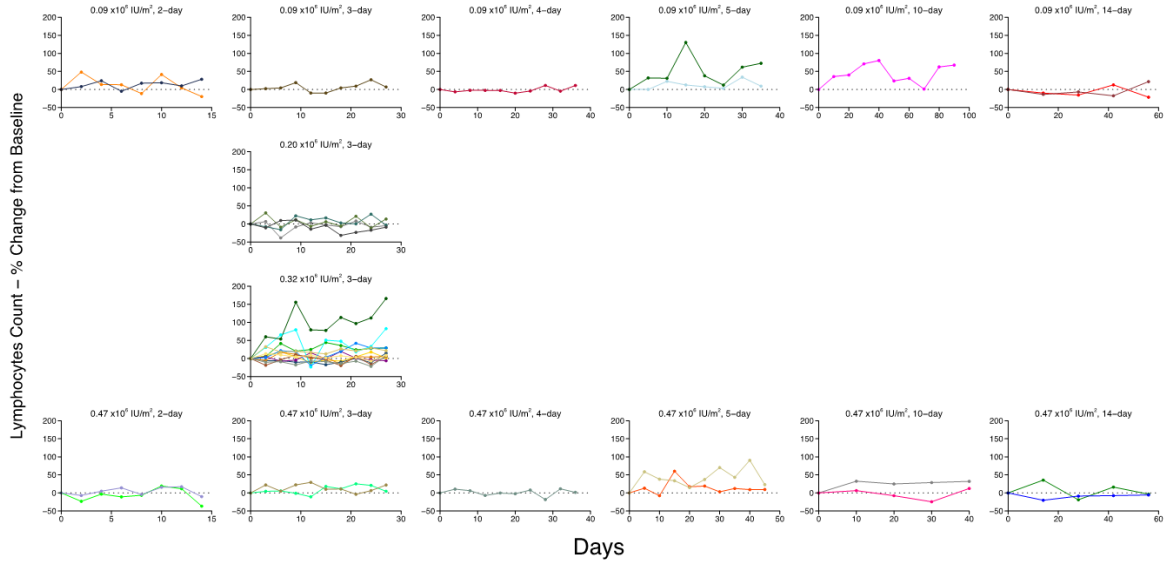


Figure S15: Percentage change of Treg % CD4 T cells in individual participants (each line) by dose and frequency (safety population)

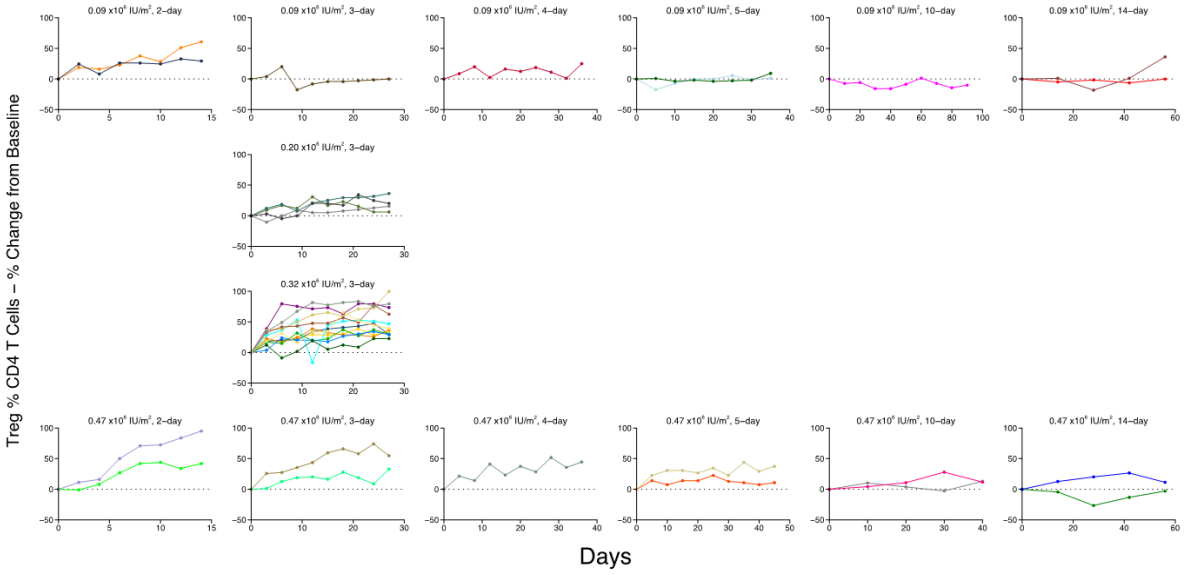


Figure S16: Percentage change of Treg absolute in individual participants (each line) by dose and frequency (safety population)

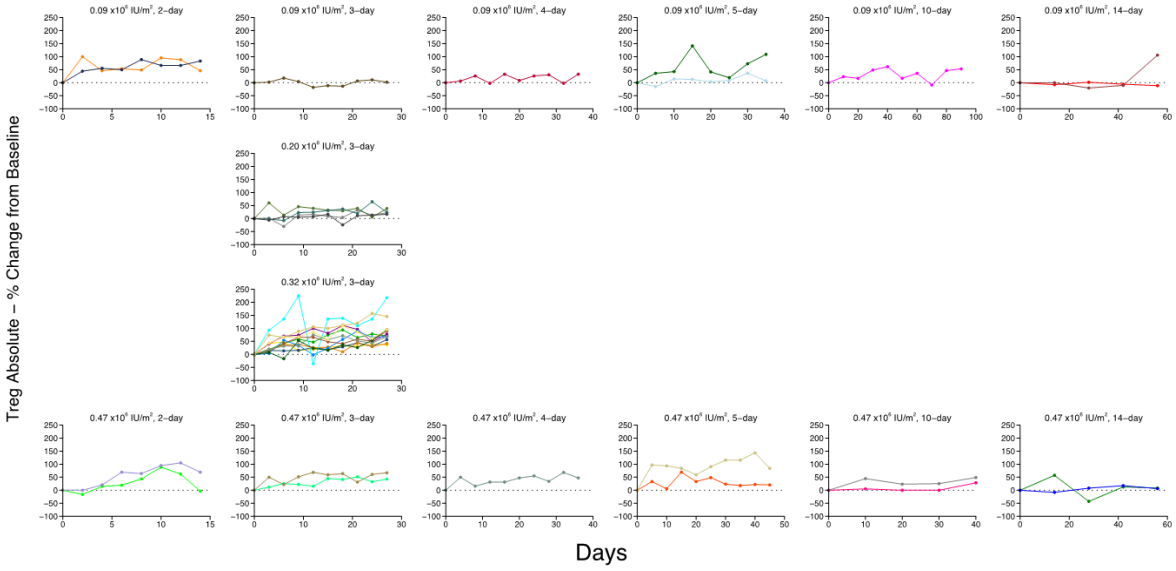


Figure S17: Percentage change of Treg CD25 Mean FI in individual participants (each line) by dose and frequency (safety population)

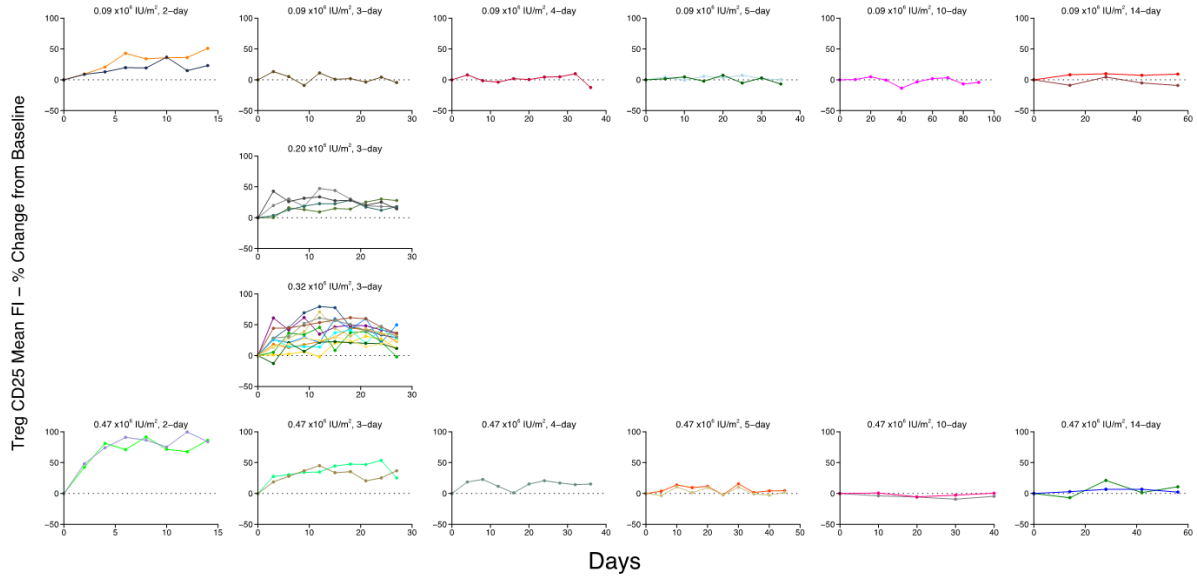


Figure S18: Percentage change of Treg CD25 MEF in individual participants (each line) by dose and frequency (safety population)

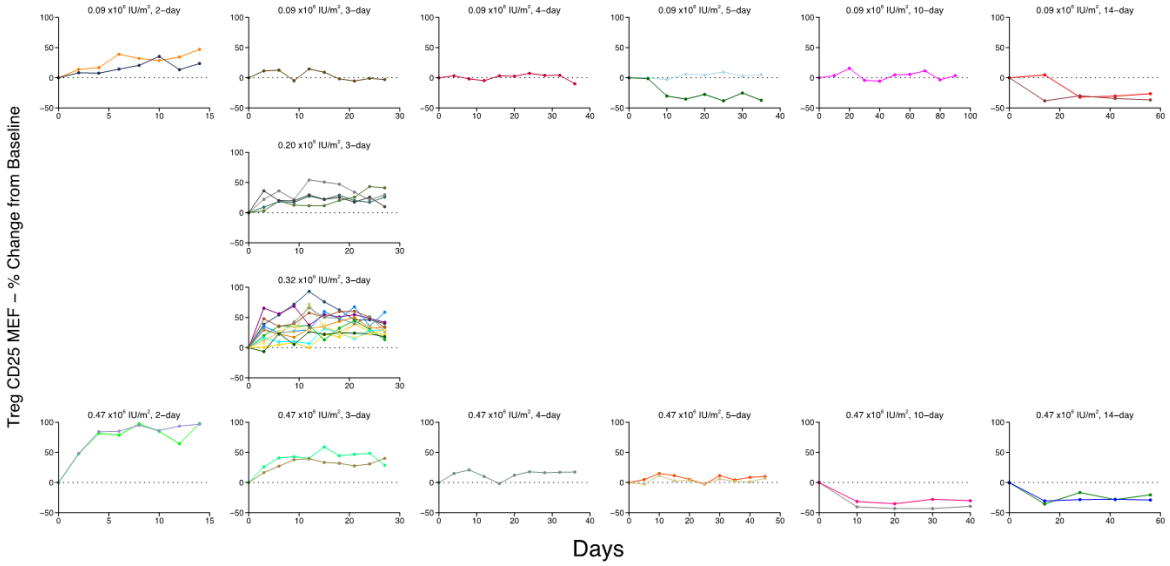


Figure S19: Percentage change of Non-Treg Naïve % in individual participants (each line) by dose and frequency (safety population)

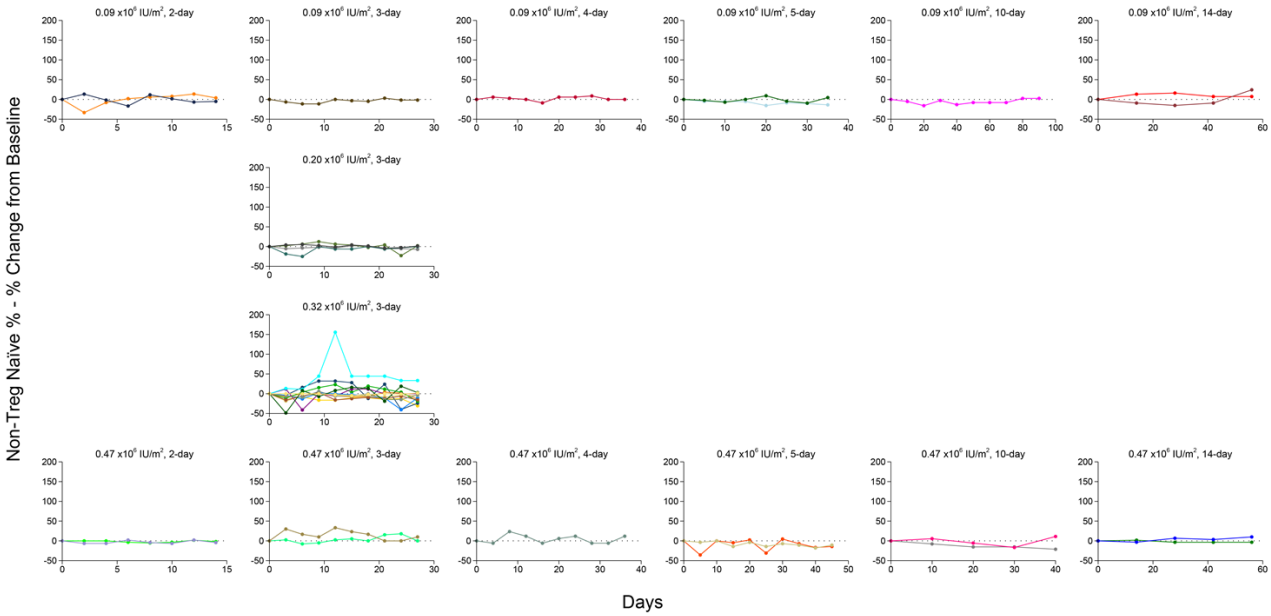


Figure S20: Percentage change of Non-Treg Naïve absolute in individual participants (each line) by dose and frequency (safety population

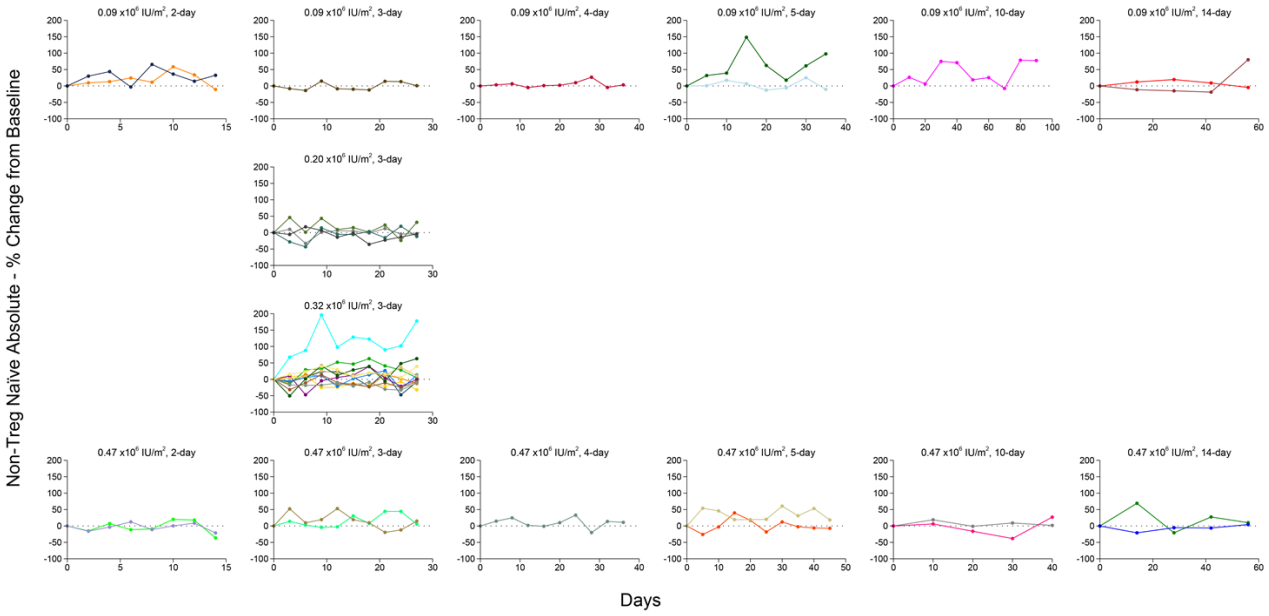


Figure S21: Percentage change of Non-Treg Temra % in individual participants (each line) by dose and frequency (safety population)

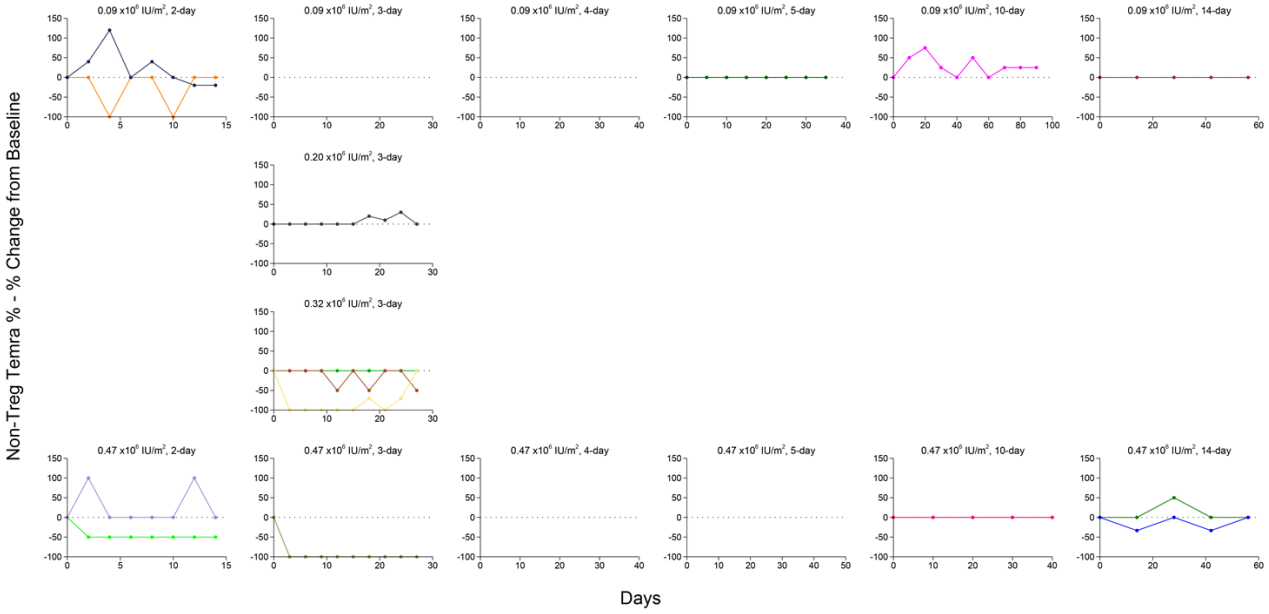


Figure S22: Percentage change of Non-Treg Temra absolute in individual participants (each line) by dose and frequency (safety population)

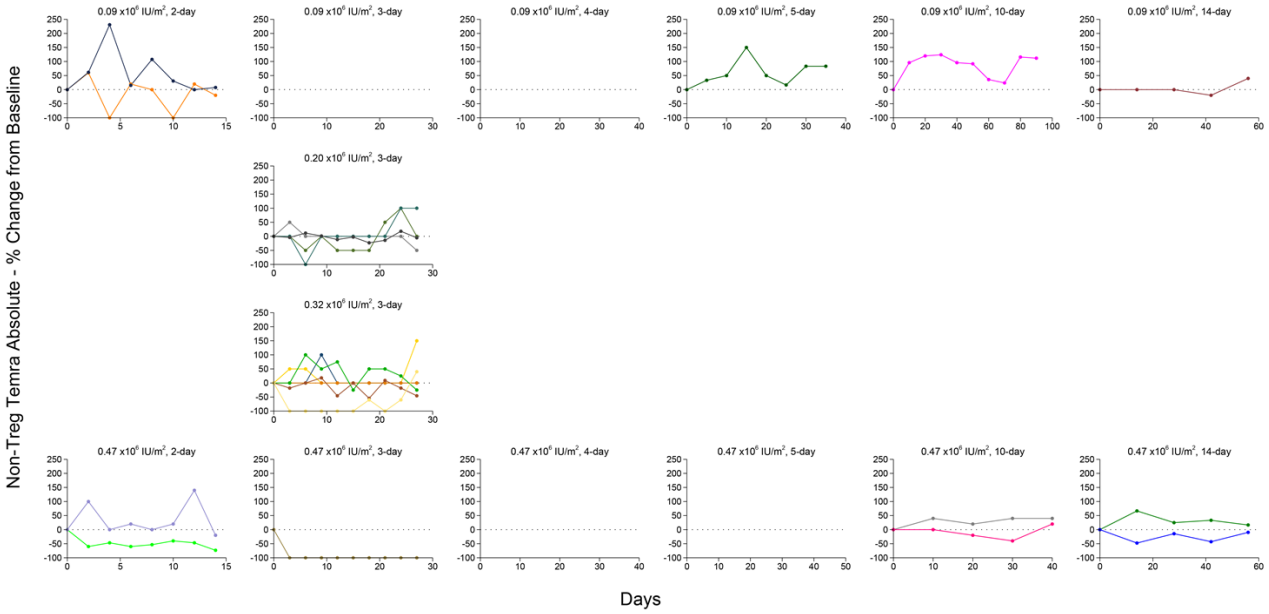


Figure S23: Percentage change of Non-Treg total memory % in individual participants (each line) by dose and frequency (safety population)

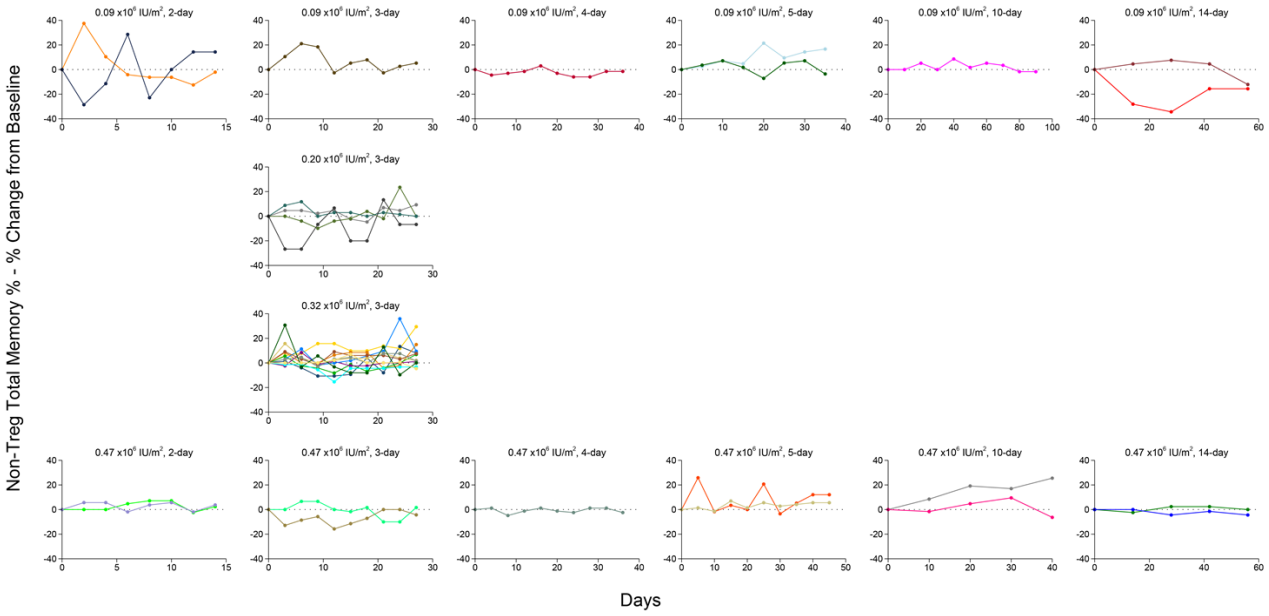


Figure S24: Percentage change of Non-Treg total memory absolute in individual participants (each line) by dose and frequency (safety population)

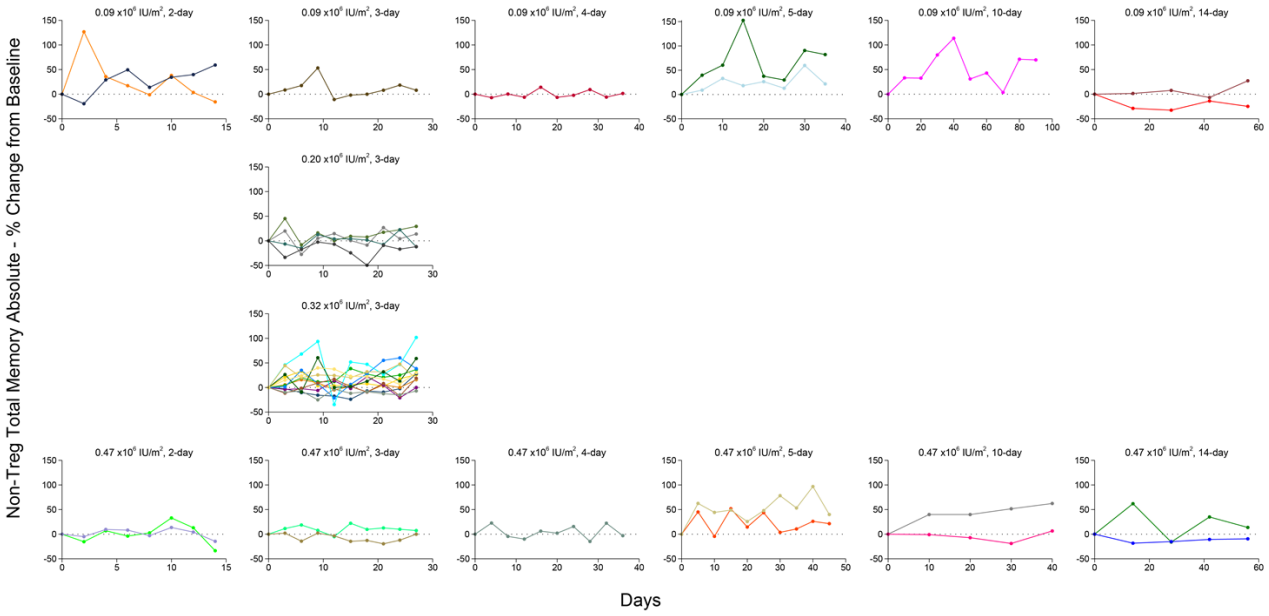


Figure S25: Percentage change of Non-Treg naïve/ memory ratio in individual participants (each line) by dose and frequency (safety population)

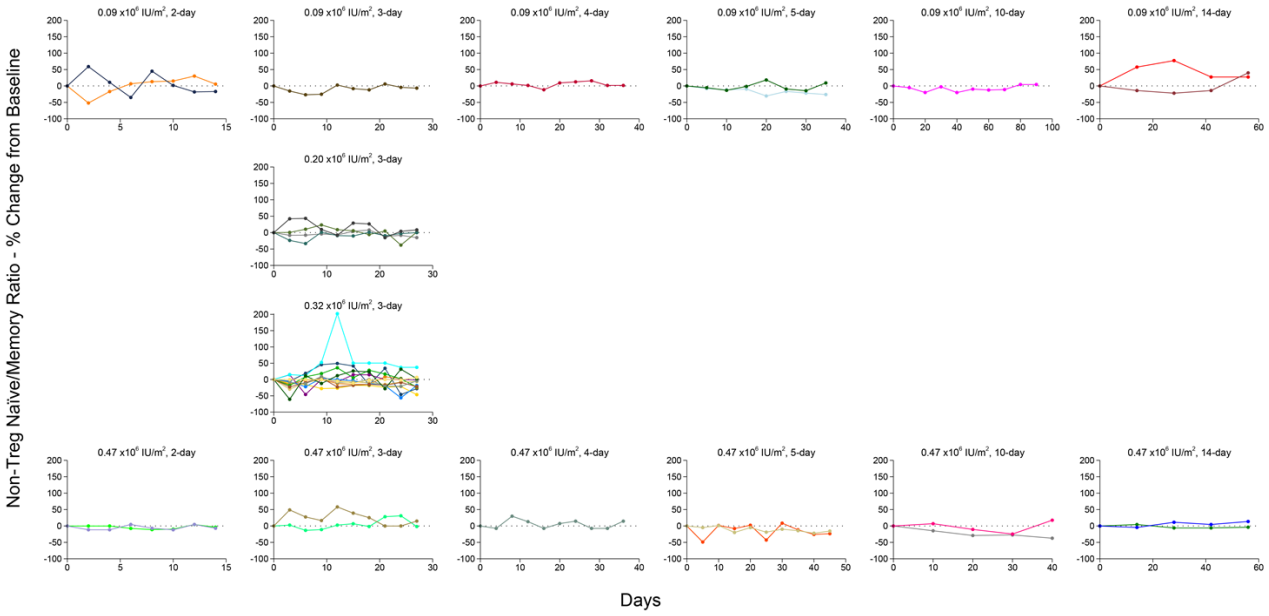


Figure S26: IL-2 levels in individual participants by dose and frequency (safety population)

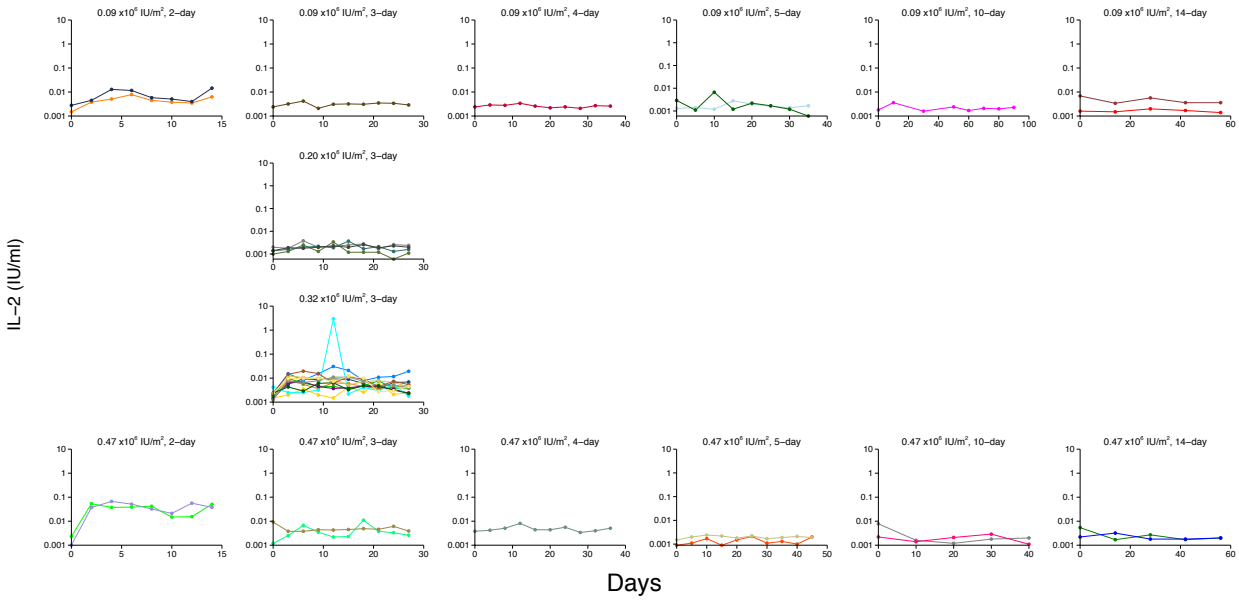
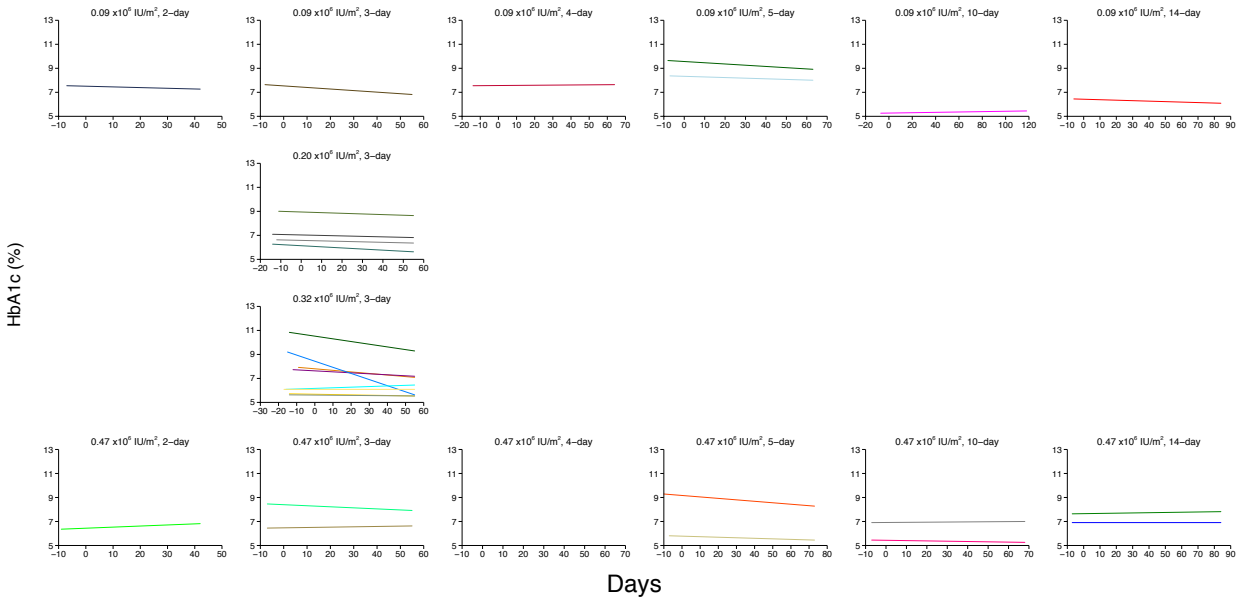


Figure S27: HbA1c (%) in individual participants (each line) by dose and frequency (analysis population)



Statistical Analysis Plan

TRIAL FULL TITLE	Adaptive study of IL-2 dose frequency on regulatory T cells in Type 1 diabetes (DILfrequency)
EUDRACT NUMBER	
SAP VERSION	2.0
ISRCTN NUMBER	40319192
SAP VERSION DATE	02/06/2017
TRIAL STATISTICIAN	James Howlett
TRIAL CHIEF INVESTIGATOR	Dr Frank Waldron-Lynch
SAP AUTHOR	James Howlett

1 SAP Signatures

I give my approval for the attached SAP entitled "Adaptive study of IL-2 dose frequency on regulatory T cell in Type 1 diabetes" dated 20 July 2016

Chief Investigator

Name: Dr Frank Waldron-Lynch
Signature:
Date: 5th May 2017

Statistician

Name: Dr Simon Bond
Signature:
Date: 6 May 2017

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3 Abbreviations and Definitions

AE	Adverse event
AIC	Akaike's Information Criterion
ALT	Alanine aminotransferase
AR	Adverse reaction
AR1	Autoregressive of order 1
AST	Aspartate aminotransferase
BSA	Body surface area
CI	Confidence interval
CRF	Case report form

CRP	C reactive protein
DBP	Diastolic blood pressure
DFC	Dose frequency committee
DP	Decimal place
ECG	Electrocardiogram
FACS	Fluorescent activated cell sorting
FBC	Full blood count
GEE	Generalised estimating equation
GGT	Gamma glutamyl transferase
HbA1c	Glycosylated haemoglobin
HBsAg	Hepatitis B surface antigen
HepC	Hepatitis C
HIV	Human immunodeficiency virus
HR	Heart rate
IL-2	Interleukin-2
MedDRA	Medical dictionary for regulatory activities
MI	Multiple imputation
NK	Natural killer
RBC	Red blood cells
RSS	Residual sums of squares
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
T1D	Type 1 diabetes
Teff	T effector cells
Treg	T regulatory cells
TSH	Thyroid stimulating hormone
ULD	Ultra-low dose
ULN	Upper limit of normal
WBC	White blood cells

4 Introduction

4.1 Preface

Type 1 diabetes (T1D) is caused by a loss of tolerance of the immune system (autoimmunity) to the body's own insulin-producing β cells of the pancreas, leading to their dysfunction and/or destruction resulting in insulin deficiency and hyperglycemia[1]. Autoreactive effector T lymphocytes (Teff) are central to disease pathogenesis and it is thought that many cases of T1D are caused by poor regulation of Teffs by CD4+ FOXP3+ T regulatory cells (Treg)[2]. The degree of β-cell destruction and insulin deficiency depends upon the age of patients at diagnosis and the duration of their disease. Children diagnosed under the age of five usually progress rapidly and completely lose their ability to make insulin, whereas those diagnosed as adolescents or adults may preserve a low level of insulin production for decades[3]. Enhancing β-cell survival and function is a key goal of T1D immunotherapy because preservation of even small amounts of endogenous insulin production can reduce the requirement for exogenous insulin, a potentially dangerous drug. Preserving a limited β-cell function can improve glucose metabolism, reduce harmful glycosylation of proteins in the body, protect against hypoglycaemia and prevent microvascular complications such as retinopathy, nephropathy and neuropathy in the long term[4-6a].

The interleukin-2 (IL-2) pathway is one of the most important genetically validated pathways with therapeutic relevance to T1D[7]. IL-2 signalling via the high-affinity, heterotrimeric IL-2 receptor which comprises CD25 (α chain), CD122 (β) and CD132 (γ), is essential for the development and maintenance of Tregs that sustain self-tolerance and prevent autoimmunity[8]. Genome-wide association studies have identified several genes in the IL-2 pathway (for example, IL2RA encoding CD25), PTPN2, IL2-IL21 and BACH2) that are associated with an increased risk of developing T1D[9]. Rare monogenic disorders in either FOXP3 (a transcription factor that drives CD25 expression and the suppressive function of Tregs) or mutations in the CD25 gene (IL2RA) itself, cause severe autoimmune syndromes including T1D[10, 10a]. Analysis and phenotyping of T cells from patients and controls with variations in IL2RA showed that reduced CD25 expression on T cells is associated with susceptibility to T1D[11-13]. Other defects in the IL-2 signalling pathway in Tregs affecting pSTAT5[11,14] and FOXP3[15] can also reduce self-tolerance to β cells. Tregs are preferentially activated by IL-2 because they constitutively express 10-fold higher levels of the heterotrimeric high-affinity IL-2 receptor than Teffs. The higher sensitivity of Tregs for IL-2 provides a potential 'therapeutic window' where it

might be possible to administer ultra-low doses of IL-2 in order to promote Treg function without stimulating a potentially unfavourable Teff response. Ultra-low dose (ULD) IL-2 is amenable for pharmaceutical intervention owing to the availability of human recombinant IL-2, (Proleukin™, also called Aldesleukin, manufactured by Novartis Pharmaceuticals UK, Limited; https://www.medicines.org.uk/emc/medicine/19322) which has extensive human safety data available. Proleukin has been used for the treatment for cancer and more recently, in trials for the treatment of the inflammatory disorders graft-versus-host-disease[16, 17] and hepatitis C-induced vasculitis[18].

We are implementing an innovative, experimental medicine strategy to deliver immunotherapy that systematically targets the key aetiological pathways in T1D[7, 19, 20]. DILfrequency and its forerunner, "Adaptive study of IL-2 dose on regulatory T cells in type 1 diabetes" (DILT1D)[20, 21] are specifically designed to analyse the effects of Proleukin on the human peripheral immune system in blood to establish the dose and frequency of administration required to preferentially enhance Tregs over Teffs. DILT1D was designed to estimate the single dose of Proleukin required to increase the frequency of Tregs by a minimum of 10% and a maximum of 20% over baseline. DILT1D also included a detailed, mechanistic analysis of the effects of Proleukin on the whole immune system, in particular any activation of the Teff arm of the immune system was investigated. DILT1D is completed and the analyses are ongoing with the results are being prepared for publication. We have used the available data to determine the initial doses to be used in the learning phase of DILfrequency.

The goal of DILfrequency is to find the optimal dose and frequency of subcutaneous Proleukin that specifically increases Treg frequency, and the amount of CD25 on Tregs, without expanding the Teff population in T1D patients. There is an urgent need for this information because previous trials of Proleukin in inflammatory diseases[16-18] and in T1D[22-26] have used relatively high doses of Proleukin. In these trials[16-18, 22-26] the high concentrations of Proleukin that were administered as loading doses have a greater potential to activate Teffs. In addition, the very large increases of Tregs observed in these trials were far beyond the physiological range and could lead to immunosuppression and increased susceptibility to infections[16-18, 22-26]. In contrast, our aim is to deliver optimal amounts of Proleukin at a precisely determined frequency that is immunomodulatory to T cells to restore the T1D immune system to a healthy homeostatic Teff-Treg balance. Previously, the

frequency of Proleukin dosing has been empirically derived from clinical experience of high dose Proleukin as immunotherapy for metastatic renal cell carcinoma[27] and human immunodeficiency virus (HIV) infection[28]. We now know that these high doses of Proleukin given in 'on then off' treatment cycles are more suitable for cancer treatment to activate Teffs and are not optimal for preserving insulin secretion and treating T1D. Results from a recent trial[29] giving T1D participant's rapamycin with 4.5 x 106 IU of Proleukin three times a week for a month was terminated prematurely because β-cell function was impaired. Rapamycin is used routinely for immunosuppression in pancreatic islet transplantation and therefore, the observed decline in β-cell function could be due to the high dose of Proleukin activating Teffs. Alternatively, Proleukin may have altered the effects of Rapamycin on β cells.

In DILfrequency we are taking a different approach: by using all of the data generated in the studies together with statistical modelling we aim to find the optimal dose and dosing-schedule for the future administration of Proleukin to attempt to preserve β-cell function in newly diagnosed T1D[21].

4.2 Purpose of the analyses

These analyses will assess the relationship of repeated dose of Aldesleukin with the change in responses as measured by fluorescent activated cell sorting (FACS).

5 Study Objectives and Endpoints

5.1 Study Objectives

5.1.1 Primary Objective

To establish the optimal dose and frequency to administer ULD IL-2 to participants with T1D in order to target a desired Treg increase, increased CD25 expression on Treg and minimal increase of CD4 Teff.

5.1.2 Secondary Objectives

To characterise the effects of repeated doses of ULD IL-2 on the immune system of participants with T1D.

5.2 Endpoints

5.2.1 Co-Primary Endpoints

The co-primary endpoints are the average of three trough values of change from baseline of CD4 Treg, CD4 T effectors and CD25 expression on Treg during treatment with ULD IL-2. Trough values are those immediately preceding drug administration and these represent the constant lowest level observed assuming steady state has been achieved.

5.2.2 Secondary Endpoints

- Treg cell number, phenotype and proliferation
- Teff cell number, phenotype and proliferation
- Natural killer (NK) cell number, phenotype and proliferation
- B lymphocyte cell number, phenotype and proliferation
- T and NK intracellular signalling
- Full blood count (FBC)
- Blood levels of IL-2, IL-10, IP-10, TNFα, soluble CD25, soluble IL-6R and high sensitive C reactive protein (hsCRP)
- Change in metabolic control (Blood glucose, HbA1c, C-peptide, insulin, proinsulin, proinsulin/ C-peptide ratio, 1,5-anhydroglucitol, autoantibody status, self-reported insulin use and self-reported glucose values)

5.2.3 Safety

Safety and tolerability parameters, including clinical history, physical examination, temperature, blood pressure, heart rate (HR), 12-lead electrocardiogram (ECG), clinical laboratory tests, and adverse event (AE) recording.

5.2.4 Exploratory Endpoints

- Genotype of T1D associated loci
- Gene expression analysis of purified lymphocyte subsets and peripheral mononucleated blood cells
- IL-2 sensitivity of T regulatory, T effector and NK subsets
- Treg suppression and T effector proliferation assays
- Antigen specific T cell assays

- Sysmex® analysis of whole blood
- Epigenetic analysis of purified lymphocyte subsets and peripheral blood
- Serum/plasma levels of cytokines, soluble receptors and inflammatory markers
- Serum/plasma and cellular metabolites
- Recruitment analysis
- Histology and immunohistochemistry of skin biopsies

5.2.5 Summary of Clinical Endpoints

A summary of the clinical FACS, FBC, biochemistry, immunology and microbiology endpoints is given in table 1.

FACS assay	Full Blood Count	Biochemistry	Immunology	Microbiology
1) CD19%	1) Basophils 10^9/L	1) Sodium mmol/L	1) TSH mU/L	1) HIV antigen/antibody
2) CD19 Total B cells *10^9/L	2) Eosinophils 10^9/L	2) Potassium mmol/L	2) Free T4 pmol/L	2) Hepatitis B surface antigen (HBsAg)
3) CD3%	3) Haematocrit L/L	3) Glucose mmol/L	3) hsCRP	3) Hepatitis C antibody
4) CD3 Total T cells *10^9/L	4) Haemoglobin g/L	4) Urea mmol/L	4) Anti-islet antibodies	
5) CD4%	5) Lymphocytes 10^9/L	5) Creatinine µmol/L	5) Anti-GAD antibodies u/ml	
6) CD4 Total *10^9/L	6) Mean Cell Haemoglobin pg	6) Albumin g/L	6) Anti-IA2 antibodies	
7) CD56%	7) Mean Cell Volume fL	7) Corrected calcium mmol/L	7) Anti-ZnT8 antibodies	
8) CD56 Total NK cells	8) Monocytes 10^9/L	8) Bilirubin (total) µmol/L	8) Anti-TPO antibodies	
9) CD8%	9) Neutrophils 10^9/L	9) Alkaline Phosphatase U/L	9) TSH Receptor antibodies iu/l	
10) CD8 Total *10^9/L	10) Platelets 10^9/L	10) ALT U/L		
11) Lymphocytes count *10^9/L	11) RBC 10^{12}/L	11) AST U/L		
12) Treg %CD4 T cells[a]	12) WBC 10^9/L	12) GGT U/L		
13) Treg Absolute *10^9/L	13) Red cell distribution width %	13) HbA1c mmol/mol		
14) Treg CD25 Mean FI[a]	14) PCT	14) C-peptide pmol/L		
15) Treg CD25 MEF	15) Mean platelet volume fL	15) Calcium mmol/L		
Non Treg gated panel		16) IL-2		
16) % CD4 T cell (NT)		17) 1,5-Anhydroglucitol		
17) Non Treg total CD4 T cells *10^9/L		18) Insulin		
		19) Proinsulin		
		20) Proinsulin/C-peptide		

18) Central Memory %		ratio		
19) Central Memory absolute *10^9/L				
20) Effector Memory %				
21) Effector Memory absolute *10^9/L				
22) Naïve %				
23) Naïve absolute *10^9/L				
24) Temra %				
25) Temra absolute *10^9/L				
26) Total memory %				
27) Total memory absolute *10^9/L				
28) Naïve / memory ratio[a]				
Treg gated panel				
29) Central memory %				
30) Central memory absolute *10^9/L				
31) Effector memory %				
32) Effector memory absolute *10^9/L				
33) Naïve %				
34) Naïve absolute				

*10^9/L 35) Temra% 36) Temra absolute *10^9/L 37) Total Memory% 38) Total Memory absolute *10^9/L 39) Naïve / Memory ratio				

Table 1: Clinical FACS, FBC, biochemistry, immunology, and microbiology Endpoints
[a] Variables from which the co-primary endpoints are derived

5.2.6 Summary of schedule of measurement of study variables

All FACS endpoints are measured at every visit between v2 and v12. 90-minute measurements are taken at v2 and 1 other dosing visit.

All FBC endpoints are measured at the same visits FACS endpoints are measured with an extra measurement taken at v1 (screening).

Biochemistry endpoints except for HbA1c, C-peptide, IL-2, 1,5-Anhydroglucitol, Insulin, Proinsulin, and Proinsulin/C-peptide ratio are measured at v1, v2, v4, v6, v8, v10, and v12.

HbA1c is measured at v1 and v12.

C-peptide is measured at every visit between v1 and v12.

IL-2 is measured at the same times as the FACS endpoints.

1,5-Anhydroglucitol is measured at v2, v9, v11, and v12.

Insulin, Proinsulin, and Proinsulin/C-peptide ratio are measured at every visit between v2 and v12.

All immunology and microbiology variables except for hsCRP are measured at v1 and v12.

hsCRP is measured at the same times as FBC endpoints.

Further clinical endpoints other than those listed in table 1 include self-assessed blood glucose and insulin use (total insulin IU/kg/day). These variables are measured daily and are recorded in participant diaries.

See Section 6.6 for a more detailed description of schedule of measurement of study variables.

5.3 Derived variables

Categorical age is defined as 1 - $18 \leq age \leq 35$, 2 - $36 \leq age \leq 65$.

The duration of disease is calculated in time at v1 by 'date of visit'-'date of diagnosis'. A categorical variable for duration of disease is also create with the following levels: 1 - duration$\leq$100 days, 2 - 100 days<duration$\leq$2 years, 3 - 2 years<duration$\leq$5 years.

BMI at v1 is rounded to 2 decimal places (DP). BMI is calculated at v12 using the following formula weight (kg)/height $(m)^2$

Where total daily dose of insulin has units of u/24h, it will be converted to u/24h/kg by dividing by weight (rounded to 2DP).

All the antibodies listed in the immunology endpoints (with the exception of anti-islet antibodies) are measured as continuous variables. However, an individual can be defined as positive or negative for a particular antibodies based on the range of the assay. The following thresholds are used to define antibody positivity:

- Anti-GAD antibodies: >5
- Anti-IA2 antibodies: >10
- Anti-ZnT8 antibodies: ≥15
- Anti-TPO antibodies: >60
- TSH receptor antibodies: >1

All antibodies are coded 1=positive, 2=negative. In addition, the number of positive antibodies at baseline and follow-up are calculated by the total number of antibody variables (anti-islet antibodies, anti-GAD antibodies, anti-IA2 antibodies, anti-ZnT8 antibodies, anti-TPO antibodies, TSH receptor antibodies) that are positive.

If at any visit, no change to the insulin regime has been made, the questions relating to insulin usage will be carried forward from the previous visit.

HbA1c is measured in mmol/mol. This is converted to % using the following formula:

(0.09148*HbA1c) + 2.152

Proinsulin/C-peptide ratio is calculated by dividing proinsulin by C-peptide.

5.3.1 Primary Endpoints

The percentage change from baseline (v2) in the three co-primary endpoints is calculated using:

Percentage change = {(measurement*100)/baseline value}-100

For each co-primary endpoint, the mean of the final three trough values (on the percentage scale) will be calculated. Both the percentage change and the mean of the three trough values are rounded to 2 DPs.

5.3.2 Repeatedly Measured Secondary Endpoints

The change and the percentage change from baseline will be calculated for all repeated measures at every visit. The change is rounded to the same precision as the raw measurement and the percentage change is rounded to 2DPs.The mean of the final three trough values will be calculated for the raw measurement, the change from baseline and the percentage change from baseline for each participant. The precision of the mean of the final three trough values will be the same as the precision of the individual trough values. Where a variable was not measured due to the trial design the mean of the trough values is calculated for the visits that are available.

5.3.3 Non-repeatedly Measured Secondary Endpoints

The change and percentage change from baseline to the final value measured are calculated for non-repeated measured secondary endpoints. Baseline is the first visit the value was measured on, either V1 or V2 depending on the variable. The change is rounded to the same precision as the raw measurement and the percentage change is rounded to 2DPs.

5.3.4 Early 90-minute Endpoint Data

For each of the endpoints where 90-minute data is measured the change, and percentage change from baseline is calculated. In this case, the baseline measure is the trough value immediately preceding the 90-minute measurement. The change is rounded to the same precision as the raw measurement and the percentage change is rounded to 2DP.

6 Study Methods

6.1 General Study Design and Plan

This is a non-randomised, repeat dose, open label, adaptive trial.

6.2 Inclusion-Exclusion Criteria and General Study Population

6.2.1 Inclusion Criteria

- T1D
- 18-70 years of age
- Duration of diabetes less than 60 months from diagnosis
- Written informed consent to participate

6.2.2 Exclusion Criteria

- Hypersensitivity to aldesleukin or any of the excipients
- History of severe cardiac disease
- History of malignancy within the past 5 years (with the exception of localized carcinoma of the skin that had been resected for cure or cervical carcinoma *in situ*)
- History or concurrent use of immunosuppressive agents or steroids
- History of unstable diabetes with recurrent hypoglycaemia
- Active autoimmune Hyper or hypothyroidism
- History of live vaccination two weeks prior to first treatment
- Active clinical infection
- Major pre-existing organ dysfunction or previous organ allograft
- Females who are pregnant, lactating or intend to get pregnant during the study
- Males who intend to father a pregnancy during the study
- Participation in a previous therapeutic clinical trial within 2 months prior to aldesleukin administration
- Donation of more than 500ml of blood within 2 months prior to aldesleukin administration
- Abnormal ECG
- Abnormal FBC, chronic renal failure (Stage 3,4,5) and/or evidence of severely impaired liver function (ALT/AST >3x upper limit of normal (ULN) at screening; alkaline phosphatase and bilirubin 2xULN at screening (isolated bilirubin >2xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%))
- Positive HBsAg or HepC serology or HIV test

- Any medical history or clinically relevant abnormality that is deemed by the principal investigator/delegate and/or medical monitor to make the patient ineligible for inclusion because of a safety concern

6.3 Subject Withdrawal Criteria

Participants may be removed from the study at their choice or at the Investigator's discretion if it is felt to be clinically appropriate. Reasons for participant withdrawal will be recorded in the paper Case Report Form (CRF) and medical notes. Data from withdrawal consented participants may be included in the trial analysis if they have received treatment. Participants who have been withdrawn will be offered if required appropriate clinical follow up at their local hospital or primary care centre. Primary reasons for withdrawal may include but is not limited to: Serious Adverse Event (SAE), AE, withdrawal of consent, lost to follow up, participant non-compliance, or trial closed or terminated. Participants who are withdrawn from the trial will be replaced as required.

6.4 Assignment

At the start of the study in the learning phase the first 12 subjects will be allocated the following doses and frequencies that are at the limits of the available combinations.

Group	Dose interval	Total number of doses	Number of participants	
			0.47×10^6 IU/m^2	0.09×10^6 IU/m^2
1	14th day	5	2	2
2	10th day	5	2	
3	5th day	8		2
4	2nd day	8	2	2

Table 2: Initial Dose/Frequency Allocations in Learning Phase

Group 1 will be the first participants to commence treatment followed sequentially by groups 2-4. A multivariate regression model of the joint distribution of Tregs, CD25 and Teffs will be developed as a function of dose and frequency. This model will provide an estimate and the standard error (SE) of the dose/frequencies that

achieve increases in Tregs and CD25 closest to the pre-specified targets. The dose/frequencies that are predicted to achieve increases closest to the targets of Tregs and CD25 will be carried forward, providing that the probability of Teffs being within a pre-specified range is sufficiently large. The doses and frequencies used may be different to those used in the learning phase if extrapolation suggests a substantially higher chance of achieving the target at such dose/frequencies.

The choices of dose/frequency will be approved by a dose frequency committee (DFC). At the end of the study, the choice of the best dose/frequency will be selected in a similar manner.

6.5 Selection of New Dose/Frequencies

This study will have four cohorts. After each of the first 3 cohorts the data will be analysed and a decision will be made as to which dose/frequencies are to be administered in the next set of participants.

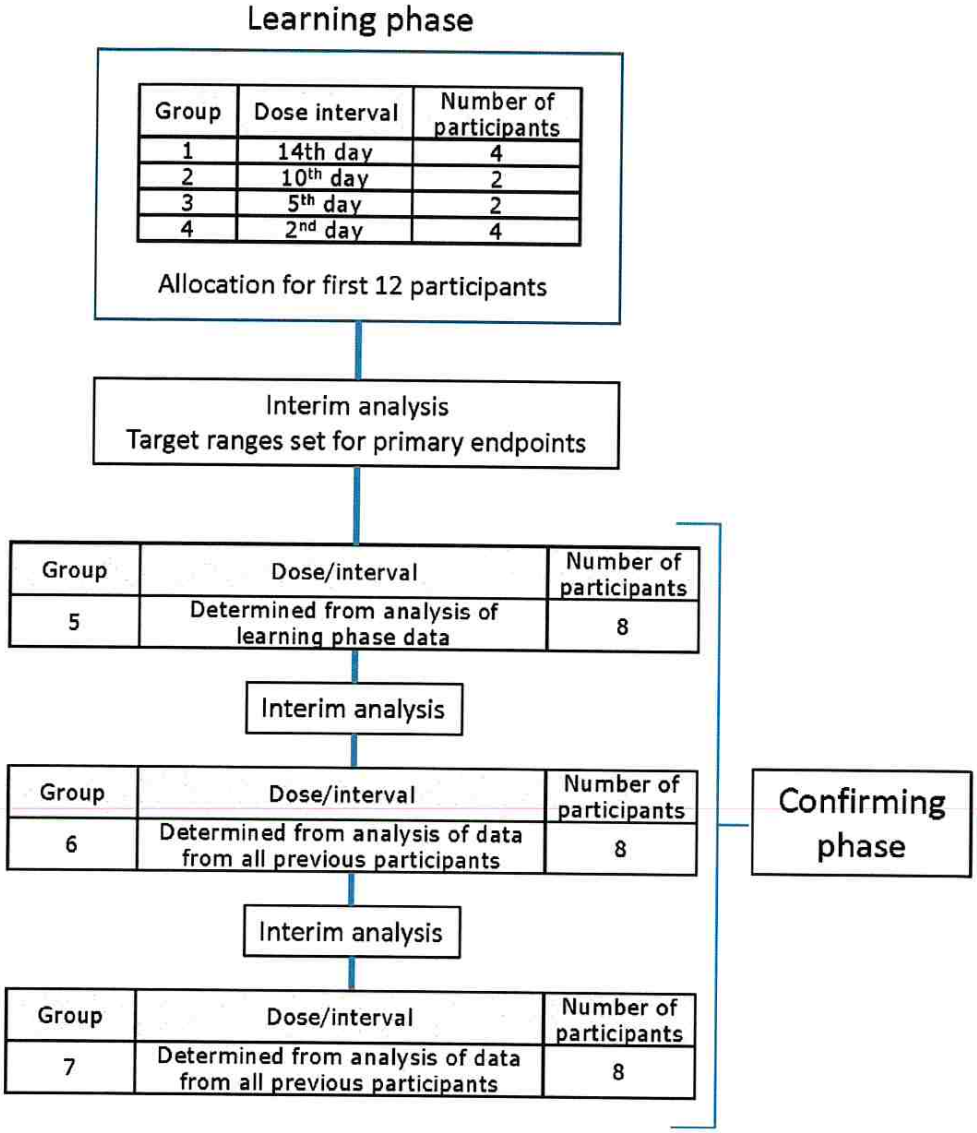


Fig 1 Dose/Frequency allocations in adaptive phase

After all participants have been dosed the data will again be analysed to determine the best dose/frequency to administer IL-2. It is hoped that as more data accrues the choice of the dose/frequency will be narrowed down and that later participants will be given the drug close to the optimal dose/frequency to confirm that this dose/frequency does effect the desired increases.

To determine the optimal dose/frequencies at the interim and at the end of the trial the data will be assessed using a multivariate regression model. The three dependant variables will be the three co-primary endpoints with the covariates being dose and frequency. Transformations of dose and frequency will be considered if it is deemed to improve the fit of the model. Transformations of dose

and frequency that will be considered to improve the model fit will be including the log of dose and/or frequency, and including a squared term for dose and/or frequency. The interaction between dose and frequency could also be included in the model. The model fits will be assessed using the Akaike's Information Criterion (AIC) and the residual sums of squares (RSS). A model will be selected from which to base the choice of dose/frequency using the AIC and RSS.

Once a model has been selected the dose/frequency that is predicted to give an increase in Tregs of 30% and an increase in CD25 of 25% can be calculated by solving the simultaneous equations. This is the predicted optimal dose/frequency to administer IL-2 to achieve the target increases. A confidence interval (CI) can be put around the optimal dose/frequency using the delta method to calculate the variance around the dose/frequency.

$$Var[x(\theta, y)] = \frac{\partial x}{\partial \theta} Var(\theta) \frac{\partial x'}{\partial \theta}$$

$\frac{\partial x}{\partial \theta} = -\left(\frac{\partial f}{\partial x}\right)^{-1} \frac{\partial f}{\partial \theta}$, evaluated at θ, x

Where $\bar{x}$ satisfies $y = f(\theta, x)$

$$y = \begin{matrix} Treg \\ CD25 \end{matrix}$$

$$x = Dose, Frequency$$

$$\theta = \beta_{0Treg}, \beta_{1Treg}, \beta_{2Treg}, \beta_{3Treg}, \beta_{0CD25}, \beta_{1CD25}, \beta_{2CD25}, \beta_{3CD25}$$

The dose/frequency that is predicted is unlikely to be a dose/frequency that is able to be administered due to the practical restrictions in the trial as the frequency is limited to integer values of day and dose is limited between 0.04-0.6x10^{6}IU/m^2/day. The Mahalanobis distance can be calculated for each dose/frequency that is able to be administered. The Mahalanobis distance is a measure of how close each dose/frequency is to achieving the target increases in Tregs and CD25. It is given by:

$$D_M = \sqrt{(x-\mu)^T S^{-1} (x-\mu)}$$

Where S^{-1} is the covariance matrix and $x-\mu$ is a 2x1 matrix containing the predicted Tregs and CD25 at each dose/frequency minus the Tregs and CD25 targets (30 and 25% respectively). A smaller Mahalanobis distance indicates a dose/frequency that is closer to achieving the target increases in Tregs and CD25.

In addition to the Mahalanobis distance, the probability that the predicted increase in Tregs, CD25, and Teffs fall within the target ranges can be calculated for each dose/frequency. The target ranges are set to be ±5% for Treg and CD25 percentage increase and -100%-∞ for Teff. Both univariate and multivariate probabilities will be calculated.

The Mahalanobis distance along with the multivariate probabilities will be used to determine the dose/frequencies that should be administered at the next cohort as well as determining the dose/frequency that provides an increase in Tregs of 30% and an increase in CD25 of 25% at the end of the trial. Particular attention will be given to the probability that the Teffs increase is within the target range. As this is a safety endpoint, if this probability falls too low, for any given dose/frequency these dose/frequencies will not be considered.

At the interim analyses the DFC will recommend which dose/frequencies should be administered to the next cohort of participants having reviewed the data.

6.6 Study Variables

Cohort 1	Screening	Administration of IL-2												Follow up
Visit Number	1	2	2/90min†	3	3-11/90min†		4	5	6	7	8	9	10	11	12
Assessments															
Informed consent	X														
Inclusion/exclusion	X														
Demography	X														
Medical history	X														
Physical exam	X	X													X
Vital signs	X	X	X	X	X		X	X	X	X	X	X	X	X	X
Fasting															
Concomitant medications	X	X		X			X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X		X	X	X	X	X	X	X	X	X
Self-assessed insulin use and glucose values**	X	X		X			X	X	X	X	X	X	X	X	X
IL-2††		X		X			X	X	X	X	X	X	X		
Clinical Chemistry	X	X					X		X		X		X		X
FBC	X	X	X	X	X		X	X	X	X	X	X	X	X	X
Clinical Immunology FACS		X	X	X	X		X	X	X	X	X	X	X	X	X
Glucose	X	X		X			X	X	X	X	X	X	X	X	X
HbA1c	X														X
hsCRP		X	X	X	X		X	X	X	X	X	X	X	X	X
Urine analysis	X														X
Pregnancy test†††	X	X													X
ECG	X														X
HIV, Hep B, Hep C	X														
Chest X ray	X														
Auto antibodies	X														X
Thyroid function tests	X														X
C-peptide	X	X		X			X	X	X	X	X	X	X	X	X
Insulin	X	X		X			X	X	X	X	X	X	X	X	X
Proinsulin	X	X		X			X	X	X	X	X	X	X	X	X
1,5-anhydroglucitol	X	X										X			X
Provision of study diary		X													
Review of diary and medication stock				X			X	X	X	X	X	X	X	X	

Inspection of injection sites			X	X	X		X	X	X	X	X	X	X	X	
Mechanistic studies															
Surface and Intracellular T cell signalling - FACS		X	X	X	X		X	X	X	X	X	X	X	X	X
Cell sorting - T cell subsets and NK		X	X	X	X		X	X	X	X	X	X	X	X	X
PSTAT5		X	X	X	X		X	X	X	X	X	X	X	X	X
RNA for expression studies		X	X	X	X		X	X	X	X	X	X	X	X	X
Epigenetics i.e FOXP3 demethylation		X	X	X	X		X	X	X	X	X	X	X	X	X
PBMC for cryopreservation		X	X	X	X		X	X	X	X	X	X	X	X	X
ELISA		X	X	X	X		X	X	X	X	X	X	X	X	X
Genotyping		X													

Cohort 2	Screening	Administration of IL-2												Follow up
Visit Number	1	2	2/90min†	3	3-11/90min†	4	5	6	7	8	9	10	11	12
Assessments														
Informed consent	X													
Inclusion/exclusion	X													
Demography	X													
Medical history	X													
Physical exam	X	X												X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fasting*		X	X	X	X	X		X		X		X		
Concomitant medications	X	X		X		X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X
Self-assessed insulin use and glucose values**	X	X		X		X	X	X	X	X	X	X	X	X
IL-2††		X		X		X	X	X	X	X	X	X	X	
Clinical Chemistry	X	X				X		X		X		X		X
FBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical Immunology FACS		X	X	X	X	X	X	X	X	X	X	X	X	X
Glucose	X	X		X		X	X	X	X	X	X	X	X	X
HbA1c	X													X
hsCRP		X	X	X	X	X	X	X	X	X	X	X	X	X
Urine analysis	X													X
Pregnancy test†††	X	X												X
ECG	X													X
HIV, Hep B, Hep C	X													
Chest X ray	X													
Auto antibodies	X													X
Thyroid function tests	X													X
C-peptide	X	X		X		X	X	X	X	X	X	X	X	X
Insulin	X	X		X		X	X	X	X	X	X	X	X	X
Proinsulin	X	X		X		X	X	X	X	X	X	X	X	X
1,5-anhydroglucitol	X	X									X			X
Provision of study diary		X												
Review of diary and medication stock				X		X	X	X	X	X	X	X	X	
Inspection of injection sites			X	X	X	X	X	X	X	X	X	X	X	

Mechanistic studies														
Surface and Intracellular T cell signalling – FACS		X	X	X	X	X	X	X	X	X	X	X	X	X
Cell sorting – T cell subsets and NK		X	X	X	X	X	X	X	X	X	X	X	X	X
PSTAT5		X	X	X	X	X	X	X	X	X	X	X	X	X
RNA for expression studies		X	X	X	X	X	X	X	X	X	X	X	X	X
Epigenetics i.e FOXP3 demethylation		X	X	X	X	X	X	X	X	X	X	X	X	X
PBMC for cryopreservation		X	X	X	X	X	X	X	X	X	X	X	X	X
ELISA		X	X	X	X	X	X	X	X	X	X	X	X	X
Genotyping		X												

Cohort 3 and 4	Screening	Administration of IL-2													Follow up
Visit Number	1	2	2/90min†	3	3-11/90min†		4	5	6	7	8	9	10	11	12
Assessments															
Informed consent	X														
Inclusion/exclusion	X														
Demography	X														
Medical history	X														
Physical exam	X	X													X
Vital signs	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Fasting*		X	X	X		X	X		X		X			X	
Concomitant medications	X	X		X			X	X	X	X	X	X	X	X	X
Adverse events		X	X	X		X	X	X	X	X	X	X	X	X	X
Self-assessed insulin use and glucose values**	X	X		X			X	X	X	X	X	X	X	X	X
IL-2††		X		X			X	X	X	X	X	X	X	X	
Clinical Chemistry	X	X					X		X		X		X		X
FBC	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Clinical Immunology FACS		X	X	X		X	X	X	X	X	X	X	X	X	X
Glucose	X	X		X			X	X	X	X	X	X	X	X	X
HbA1c	X														X
hsCRP		X	X	X		X	X	X	X	X	X	X	X	X	X
Urine analysis	X														X
Pregnancy test†††	X	X													X
ECG	X														X
HIV, Hep B, Hep C	X														
Chest X ray	X														
Auto antibodies	X														X
Thyroid function tests	X														X
C-peptide	X	X		X			X	X	X	X	X	X	X	X	X
Insulin	X	X		X			X	X	X	X	X	X	X	X	X
Proinsulin	X	X		X			X	X	X	X	X	X	X	X	X
1,5-anhydroglucitol	X	X										X			X
Provision of study diary		X													
Review of diary and medication stock				X			X	X	X	X	X	X	X	X	
Inspection of injection sites			X	X		X	X	X	X	X	X	X	X	X	

Mechanistic studies														
Surface and Intracellular T cell signalling – FACS		X	X	X	X	X	X	X	X	X	X	X	X	X
Cell sorting – T cell subsets and NK		X	X	X	X	X	X	X	X	X	X	X	X	X
PSTAT5		X	X	X	X	X	X	X	X	X	X	X	X	X
RNA for expression studies		X	X	X	X	X	X	X	X	X	X	X	X	X
Epigenetics i.e FOXP3 demethylation		X	X	X	X	X	X	X	X	X	X	X	X	X
PBMC for cryopreservation		X	X	X	X	X	X	X	X	X	X	X	X	X
ELISA		X	X	X	X	X	X	X	X	X	X	X	X	X
Genotyping		X												

† Two venepunctures will be carried out 90 minutes (90min) post administration of aldesleukin. One at Visit 2 and one at a visit between V3–11. For the 1st cohort these took place at V2, and V3 (shorter frequencies) or V4 (longer frequencies). In the 2nd cohort these took place at V2 and V3. In subsequent cohorts one took place at V2 and the other could be at any visit from V3–11.

†† In the first cohort IL-2 dosing occurred from V2–9 for shorter frequencies and V2–10 (every other visit) for longer frequencies. From cohort 2 onwards, IL-2 was given every visit from V2–11 at every frequency.

††† Additional pregnancy tests took place every 25–30 days during the dosing period.

* Fasting visits changed from cohort 2 to 3/4 as 90 minutes blood sampling was moved from V3 (cohort 2) to V11 (cohort 3/4)

** Self-assessed insulin use and glucose values were measured on a daily basis throughout the whole study

7 Sample Size

This study is not designed to formally test a hypothesis in a confirmatory fashion and so a formal power calculation in inappropriate. The sample size of 36 will be achievable within the proposed time scale given the recruitment rates established within the study site for previous studies. Simulations indicate that 36 patients will provide valuable information under scenarios that represent a scientifically plausible and clinically relevant relationship between dose/frequency and the co-primary endpoints. Such information will be able to distinguish between effective and ineffective dose/frequencies to use in subsequent clinical studies.

8 General Considerations

8.1 Timing of Analyses

The final analysis will be performed when 36 participants have completed v12.

8.2 Delegation of Analyses

Clinical data from the DIL frequency clinical database (primary and clinical endpoints) will be primarily analysed by the Cambridge Clinical Trials Unit statistical team. Data from the DIL frequency mechanistic database (secondary and exploratory) will be primarily analysed by the DIL frequency statistics team.

8.3 Analysis Populations

8.3.1 Safety Population

All participants who received any IL-2 regardless of whether the co-primary endpoints were observed. Participants who drop out prior to receiving any IL-2 are excluded. The safety analyses will be performed on this population.

8.3.2 Evaluable Population

All participants who received all repeat doses of IL-2, where the primary endpoint was observed.

8.3.3 Analysis Population

The analysis population is a subset of the evaluable population. It is defined as all participants who completed the repeat dosing schedule of IL-2, who completed the v12 follow-up visit and where the co-primary endpoints were adjudged to have

been in steady state. Inclusion in this population requires the co-primary endpoints to be recorded at each visit (v2-v12) and for steady state to have been achieved in all co-primary endpoints. This is the population on which the primary analysis will take place.

8.3.4 Determination of Analysis Population

To be included in the analysis population a participant must have Treg, CD25, and Teff measurements recorded at all dosing visits and to not have missed a dose. Any participant where any of the co-primary endpoints are missing are excluded from this population. In addition, to be included in this population the co-primary endpoints had to be adjudged to have been in steady state. The DFC will consider the profiles of each participant individually and make a judgement as to whether each participant has achieved steady state. The DFC will be made up of a minimum of one clinician, one statistician, and one scientist. Steady state will not be achieved if the trough values of percentage increase of Tregs, CD25 or Teffs have an upward or downward trend at the end of the dosing schedule.

It is also thought that some AEs could affect the values of the co-primary endpoints. Having an AE does not exclude a participant from the analysis population but a further analysis will be considered where participants who had an AE thought to affect the values of the co-primary endpoints will be excluded. Three health care professionals independently assessed whether an AE was likely to be due to a viral infection. A majority of the blinded reviewers was needed to determine whether an AE was viral.

8.4 Covariates and Subgroups

The covariates to be included in the regression model are dose and frequency. In addition, transformations of dose and frequency may be included if it improves the fit of the model. For example, squared frequency may be included as a covariate. RSS and AIC will be used in determining how dose and frequency should be included in the model.

8.5 Missing Data

There are not expected to be substantial drop-out levels. When a participant is withdrawn from the study part way through the dosing schedule, they will be replaced so that 36 participants are recruited.

Participants that miss a dosing visit but the final three trough values of the co-primary endpoints are present will be excluded from the analysis population and included in the evaluable population.

Participants where the co-primary endpoints are adjudged not to be in steady state will be excluded from the analysis population but included in the evaluable population.

If >5% of data are missing in the co-primary endpoints, multiple imputation (MI) will be used.[30] The number of imputations used will be equal to the percentage missingness (e.g. if 8% of participants are missing co-primary endpoint data, we will use 8 imputations).

MI will also be performed on missing outcome data for all secondary analyses in a similar way to the primary analysis.

If the date of diagnosis is partially missing and only the month and year are present, the day of diagnosis is assumed to be the 15th.

8.6 Interim Analyses and Data Monitoring

8.6.1 Purpose of Interim Analyses

The purpose of the interim analyses is to recommend dose/frequencies for the next cohort of patients.

Prior to the DFC meeting, protocol violators will be reported to the statisticians and analysis to assess doses and frequency will be done on a per-protocol population. The profile plots of the repeated measurements of the raw Treg, CD25, and Teff values will be assessed by statistical analysis and reviewed by the DFC to determine whether steady state has been achieved.

At the first interim analysis the point targets and target ranges of the co-primary endpoints is to be fixed. This decision will be made by a DFC. The probability of achieving the target at each of the dose/frequencies observed, along with any potential new dose/frequencies, will be documented for each set of modelling assumptions. The plausibility of the modelling assumptions generated by the study statistician will be reviewed by the DFC.

The choice of dose/frequencies to allocate will be determined by the DFC according to section 5.5.

8.6.2 Planned Schedule of Interim Analyses

The first interim analysis will occur after 12 participants have been recruited and have completed v11. Two further interim analyses will occur after 20 and 28 participants have completed v11 respectively.

8.6.3 Scope of Adaptations

The choice of dose/frequencies to assign to the next cohort will be decided by the DFC. The choice will be made after consideration of the analyses, but will not be bound by formal decision rules. The choice of dose will always lie below the maximum of $0.6x10^6 IU/m^2/day$.

The DFC will have the option to recommend to the Trial Steering Committee to amend or stop the study if relevant information, internal or external to the trial, arises.

8.6.4 Documentation of Interim Analyses

All data, code and reports used at the interim analyses will be stored on the MRC BSU network as well as the NHS computer system.

8.7 Multi-centre Studies

(ICH E3;9.7.1, 11.4.2.4. ICH E9; 3.2)

This is a multi-centre trial with co-ordination of the clinical trial carried out by the Cambridge Clinical Trials Unit, Cambridge University Hospitals NHS Foundation Trust. The primary study site will be located at Addenbrooke's Hospital initially though other study centres may be established if required to ensure adequate recruitment of participants.

8.8 Multiple Testing

(ICH E3; 9.7.1, 11.4.2.5. ICH E9; 2.2.5)

This is not a confirmatory study, we will not consider multiple testing although we do acknowledge that any finding relating to secondary endpoints will be treated as hypothesis generating.

9 Summary of Study Data

Summary statistics will be provided for all co-primary and secondary endpoints. Continuous variables will be summarised using mean, standard deviation (SD), median, max, min; categorical and binary variables will be summarised using frequency tables reporting "x/n (p%)" format.

In addition, 2 appendices will be produced. The first will provide the raw measurement, change, and percentage change for each participant at each visit for all FACS and FBC endpoints, hsCRP, IL-2, insulin, proinsulin, proinsulin/C-peptide ratio, glucose, HbA1c, all antibody endpoints (raw measures rather than binary positive/negative), insulin total daily dose, and 1,5-anhydroglucitol. 90-minute data will also be included. The second will provide summary statistics (mean, SE, CIs) for the raw measurement, change, and percentage change for each of the variables in the first appendix broken down by dose and frequency. The SE and CIs will only be reported if there are at least 4 participants in a group.

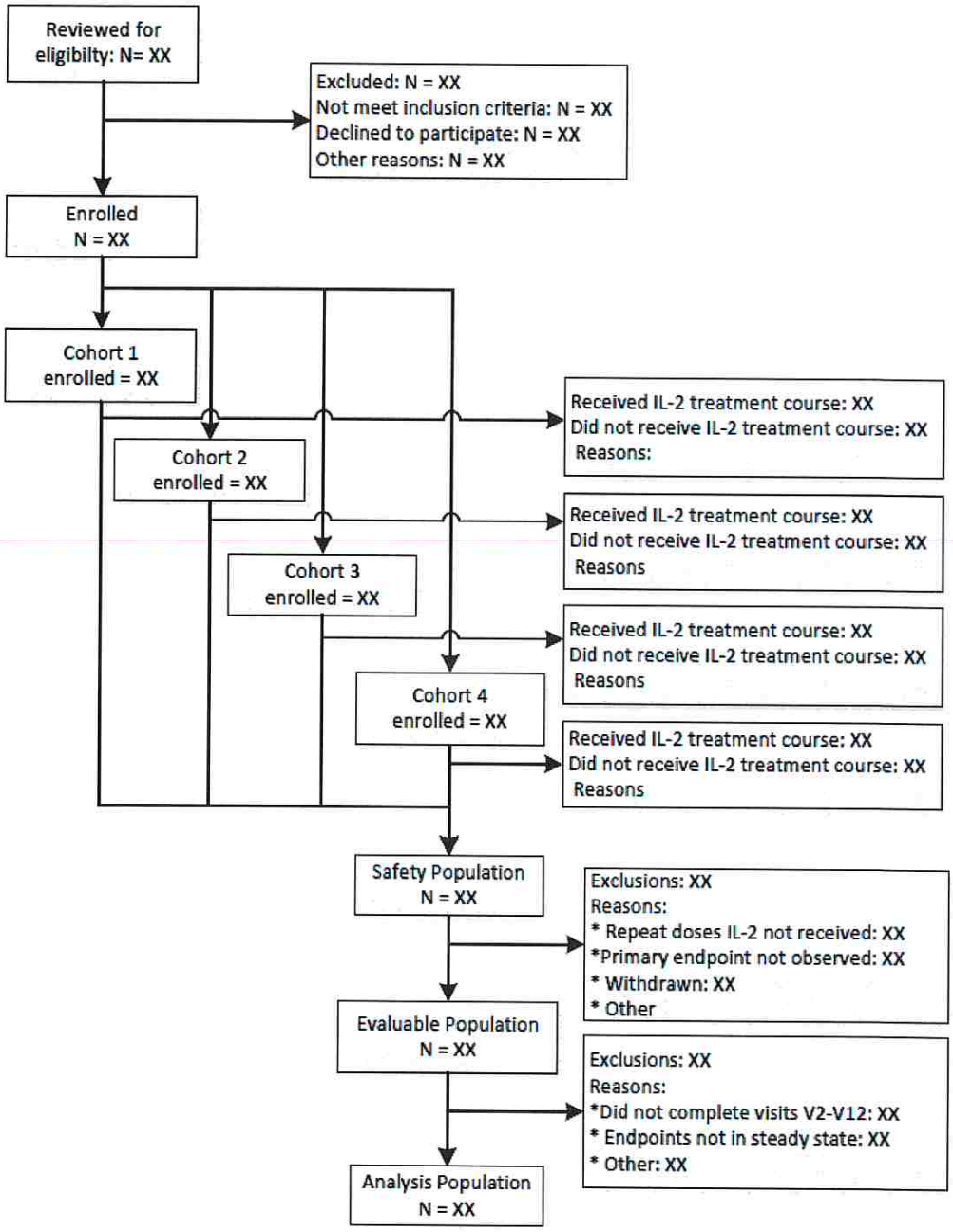
Scatter plots will be presented summarising the marginal relationships between the 3 co-primary endpoints, dose and frequency.

Profile plots of the repeated measurements of the raw Tregs, Teffs, CD25 values will be presented for all the patients.

The data will be ordered by subject and visit number.

9.1 Subject Disposition

The CONSORT diagram[31] will be completed. The withdrawal information page of the CRF will be used to determine when and if a subject was withdrawn.



9.2 Protocol Amendments

The number of dosing visits for all participants was extended to include all visits between v2 and v11 (inclusive) following the first interim analysis. The second 90 minute measurement visits was changed for cohorts three and four to be between v3-v11.

9.3 Demographic and Baseline Variables

The following variables will be summarised in the "Safety", "Evaluable", "Analysis", populations:

- Demographics: age, categorical age, gender, ethnicity, age at diagnosis, disease duration, date of diagnosis, symptoms prior to diagnosis and duration of symptoms in weeks before diagnosis (polydipsia, polyuria, weight loss, and ketoacidosis), overall pattern of symptoms prior to diagnosis
- Medical history
- Physical examination (cardiovascular, respiratory, gastro-intestinal, and neurological)
- Vital signs/physical measurements (height, weight, BMI, body surface area (BSA), systolic blood pressure (SBP), diastolic blood pressure (DBP), HR, temperature, blood glucose
- Insulin usage record (regimen and total daily dose)
- FACS
- FBC
- Biochemistry
- Immunology
- Microbiology
- Pregnancy test
- Urinalysis
- Chest X-ray
- ECG
- Dosing level and schedule
- Concomitant medications

Tables describing the demographic and baseline variables will be created, and summary statistics will be reported.

9.4 Concurrent Illnesses and Medical Conditions

Any relevant medical history and concomitant medication will be listed and summarized. This will be included in a separate appendix.

10 Efficacy Analyses

The data at baseline will be summarised using summary statistics. All responses measured repeatedly will be explored graphically, by plotting each response against elapsed days for each patient (profile). The profiles of the individuals will be grouped by dose and frequency.

10.1 Primary Efficacy Analysis

The aim of the primary analysis is to identify the dose/frequencies that achieve a Treg increase (30%) and a CD25 increase (25%), while minimising the Teff increase.

Initially the profiles of each of the co-primary endpoints will be plotted at each dosing visit. The visit of the first dose (v2) will be day 0. The mean of the three trough values for each endpoint will be summarised and then plotted against dose and frequency in a 3D plot. The relationship of the co-primary endpoints with dose and frequency will be explored by fitting a number of candidate models. The candidate models include linear, quadratic (dose and/or frequency), log (dose and/or frequency). For each model, its estimated coefficients with their SE will be reported. The AIC and RSS for each model will be reported as measures of adequacies of fit. The residual values of each model against its predicted values will be plotted, as well as a quantile-quantile plot of its residuals. The proposed target dose/frequency of each model with their SEs and with a 95% CI will be reported. The target dose/frequency will be selected as described in section 7.5.1. The 5 best 'allowable' dose/frequencies (i.e. $0.04 \leq dose \leq 0.6$, $2 \leq frequency \leq 14$ where the precision of dose is 2DP and frequency must be integer days) will be reported based on the Mahalanobis distance and joint probability for each analysis model. Contour plots showing the joint probability of the increase in Treg and CD25 being within the target ranges at each dose/frequency will be produced.

The primary analysis will be conducted both using the "Analysis Population" and the "Evaluable Population".

10.2 Secondary Efficacy Analyses

10.2.1 Repeatedly Measured Secondary Endpoints

Profiles for all repeated secondary measured endpoints will be plotted in a similar manner to the co-primary endpoints. This will be done for the raw measurements, change, and percentage change. The mean of the three trough values for each endpoint will be summarised and then plotted against dose and frequency in a 3D plot. The 3D plot will not be included in the final report but will be added to a separate appendix to the report. These responses will be analysed using a similar regression model to the co-primary endpoints. However, a univariate linear regression models will be used rather than a multivariate regression model. The linear regression will include the same parameters used in the model that was selected for the primary analysis. The coefficients and p-values will be reported. Further plots of each endpoint against frequency will be plotted and included in an appendix to the report. The plots will include a predicted line and CI given the dose is set to be the estimated best dose from the primary analysis. A global F-test will assess whether any of the dose and frequency parameters in the model are not equal to zero. This will be reported along with the proportion of the variability in the outcome explained by the model (R^2).

In addition, a generalised estimating equation (GEE) model will assess the effect of time on each of the responses measured repeatedly. All the measurements from visits where IL-2 is administered will be included in the model. The choice of working correlation will have an autoregressive of order 1 (AR1) structure. It will include fixed effects for dose, interval and time (in days). The AR1 structure of the covariance matrix allows for measurements on the same individual to be correlated as well as observations closer in time. The unbalanced nature of the data due to the participants having different dosing schedules, means that the model assumes that the correlation between observations at visit 3 and 5 (or any combination of visits) is equal across all participants even if the number of days between the observations was different. Robust SEs will be calculated. It is also of interest to assess the impact of fasting in cohorts 2, 3 and 4. Therefore, fasting will be included in the model as a time-varying covariate. The coefficients and p-values for the effects of dose, interval, time, and fasting will be reported. If there is a significant effect (p-value <0.05), further analysis using more complex models may be warranted to explore the relationship further. However, any findings will be reported with caution.

10.2.2Non-repeatedly Measured Secondary Endpoints

Non-repeated measure endpoints will be analysed in a similar way to the repeatedly measured secondary endpoints with the endpoint calculated at the final measurement rather than the mean of the final three trough values. Longitudinal models will not be fitted for these endpoints.

10.2.3 Early 90-minute Endpoint

For each endpoint where 90-minute data is collected, a linear mixed effects model will be fitted. The outcome will be the difference between the 90-minute measurement and the pre-dose measurement and the covariates included in the model will be dose, frequency and categorical visit number. The coefficients, SEs, p-values and 95% CIs for the intercept, dose, frequency and visit will be reported. The models will include a random intercept with the participant being the grouping variable.

10.2.4 Self-assessed Insulin and Glucose

Self-reported insulin use and self-monitored blood glucose are also measured daily over the course of the study in participant diaries. Insulin use has 1 record per day per subject over the course of the study record as total daily dose of insulin per kg. Self-monitored glucose is measured multiple times per day (breakfast, lunch, supper, bedtime, other) over the course of the study. Only the records corresponding to breakfast, lunch, supper and bedtime will be used for these analyses.

Similar mixed-effects models to those used for the repeatedly measured outcomes, will be used to analyse the effect of time on self-reported insulin and self-monitored glucose.

Summary statistics of self-reported insulin use and self-monitored blood glucose will also be tabulated. The statistics will be broken down into time periods as well as the overall values being reported. The time periods used will be dose 1-before dose 2, dose 2-before dose 3,...,dose 9-before dose 10, final dose onwards.

10.2.5 Antibodies

There are 4 possible states of antibody positivity: negative at baseline and follow-up, negative at baseline and positive at follow-up, positive at baseline and negative at follow-up, and positive at baseline and follow-up. The number and percentage of participants in each state will be listed for each of anti-islet, anti-GAD, anti-IA2, anti-ZnT8, anti-TPO, and TSH receptor antibodies. In addition, the p-value from a

paired chi-squared test (McNemar's test) will be reported to test for a change in antibody positivity for each endpoint.

10.2.6Correlation Analyses

The correlation coefficient between C-peptide and 1,5-anhydroglucitol, HbA1c, glucose, and proinsulin at baseline will be reported along with the 95% CI. Baseline is v2 for the 1,5-anhydroglucitol, glucose and proinsulin correlations but v1 for the correlation with HbA1c. Only visits where the participant fasted will be used.

10.2.7 Fasting

For each visit the number and proportion of participants that were supposed to fast along with the number and proportion of participants who actually fasted will be tabulated.

All secondary analyses will be performed on both the "Evaluable" and "Analysis" populations.

10.3 Exploratory Efficacy Analyses

We wish to note that additional exploratory and mechanistic assays are being conducted by DILfrequency scientists and clinicians. Their analysis will be conducted by DILfrequency statisticians.

11 Safety Analyses

11.1 Safety Endpoints

The baseline data of the safety endpoints will be summarised.

The profiles of the clinical FACS, FBC, biochemistry, and hsCRP variables will be generated.

Paired t-tests will be conducted for testing the null hypothesis that measurement of visit 12 is no different from the measurement of visit 2 (or screening visit where available) for all safety endpoints (FACS, FBC, biochemistry, TSH, free T4, hsCRP).

The following variables will be summarised at v12:

- Physical examination (cardiovascular, respiratory, gastro-intestinal, and neurological)

- Vital signs/physical measurements (height, weight, BMI, BSA, SBP, DBP, HR, temperature, blood glucose
- Insulin usage record (regimen and total daily dose)
- FACS
- FBC
- Biochemistry
- Immunology
- Pregnancy test
- Urinalysis
- ECG

11.2 Extent of Exposure

Repeat doses of IL-2 will be administered and IL-2 levels will be measured in blood. The minimum dose that can be administered is $0.04x10^6 IU/m^2/day$ with the maximum dose $0.6x10^6 IU/m^2/day$

11.3 Adverse Events (AE)

All AEs will be recorded and summarised at the end of the trial and then these will be categorised according to the definitions in MedDRA coding. When calculating the incidence of AEs, each participant will only be counted once and any repetitions of AEs will be ignored; the denominator will be the total population size.

11.4 Adverse Reaction (AR) to a Medicinal Product

Any AR will be recorded and summarised at the end of the trial according to the categories defined in MedDRA coding. When calculating the incidence of ARs, each subject will only be counted once and any repetitions of ARs will be ignored; the denominator will be the total population size.

Expected ARs will also be listed. These include: systemic symptoms, localised reaction at site of injection, generalised gastrointestinal symptoms, haematopoiesis, and thyroid dysfunction.

11.5 Deaths, Serious Adverse Events and other Significant Adverse Events

The only expected SAEs are severe hypoglycaemia, hyperglycaemia, and ketosis. All SAEs/SARs will be listed.

11.6 Other Safety Measures

Vital signs and any other records included in the Medical Notes sections of CRFs or source data will be documented and summarised at the end of the trial.

11.7 Reporting of AEs

For each AE or AR, the start and end time, a description, the higher level group term MedDRA code, the outcome, the seriousness, the causality (drug related or not), and the severity will be listed.

In addition, the following tables will be produced with one with the denominator as number of AEs and again where the denominator is the number of participants in the safety population:

- Unexpected adverse events
    - By severity (severe, mild, moderate)
    - Most common (>10%) - PT MedDRA Code
    - By relatedness (unrelated: not related/unrelated/unlikely; related: possibly, probably, almost certainly)
- Expected adverse events
    - By severity (severe, mild, moderate)
    - Most common (>10%) - PT MedDRA Code
    - By relatedness (unrelated: not related/unrelated/unlikely; related: possibly, probably, almost certainly)
- Unrelated adverse events
    - By severity (severe, mild, moderate)
    - Most common (>10%) - PT MedDRA Code
    - By expectation (expected or unexpected)
- Related adverse events
    - By severity (severe, mild, moderate)
    - By PT group MedDRA Code
    - By expectation (expected or unexpected)
    - By severity and cohort
    - By PT group MedDRA Code and cohort
    - Drug related injection site reactions per number of doses
    - Drug related injection site reactions per number of doses per cohort
    - Hypoglycaemic event

For the tables that are produced for the subset of 'related' AEs, the AEs where the relatedness is recorded as 'possibly', 'probably', or 'almost certainly' are used. Hypoglycaemia information is taken from the participant diary data and is defined as glucose <3mmol/l.

12 Figures

A graph showing whether participants adhered to the dosing schedule will be generated at the end of the trial. This will show how close the actual visit dates were to the schedule for dosing visits.

Graphs that illustrate the dosing procedure of the trial (dose/frequency against patient number) will be generated at the end of the trial.

Graphs of the time-course (profile) of the patients for all variables that longitudinal data points have been collected will be plotted. The time-courses will be divided by patient, and by dose/frequency. Profiles will be split into fasting/non-fasting visits for metabolic endpoints.

Graphs showing the values of the co-primary endpoint will be plotted against dose and frequency. These could be 3d to capture both the effect of dose and frequency on each of the co-primary endpoints.

Plots of the secondary endpoints against dose and frequency will be generated.

Bar plots showing the mean change from baseline of the endpoints and mean change from baseline of the 90-minute data by dose and frequency may be produced. SE bars will be added where N>=4.

13 Reporting Conventions

P-values ≥0.00001 will be reported to 5DP; p—values less than 0.00001 will be reported as "<0.00001". The mean, SD, and any other statistics other than quantiles will be reported to 1 DP greater than the original data. Quantiles will use the same number of DPs as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

14 Technical Details

Protocol version 4.1 and CRF version 3 were used to develop this document. Software to be used may include Stata version 13 and 14, and R version 3.2.

15 Summary of Changes to the Protocol

Updated so dosing visits are all visits from v2-v11 for all frequencies to allow each participant more opportunity to reach steady state.

Updated, so that participants were required to fast at some visits.

Updated the visits at which the 90 minute measurement could be taken to include all dosing visits.

16 Summary of Changes to the SAP

This section documents the changes that have been made to the SAP v1.0.

Added Proinsulin/C-peptide ratio to the table in section 5.2.5 and removed anti-HBc.

Included details in section 5.2.5 on when each endpoint is recorded.

Added more details to section 5.3 explaining how to derive categorical age, diagnosis duration, BMI, total daily dose of insulin, antibodies positive/negative, insulin usage information, HbA1c, primary endpoints, secondary endpoints, and 90 minute data.

Added a sentence to section 6.5 allowing the interaction between dose and frequency to be included in the primary analysis model. Added a sentence about how the model will be determined. Added a sentence stating the target ranges.

Changed 'subjects' to 'participants' in section 8.3. Removed 'and were not withdrawn from the study on clinical grounds' from section 8.3.2.

Rewording sentence to describe steady state in section 8.3.4. Change 'medical reviewers' to 'health care professionals'.

Added a sentence to section 8.5 explaining the imputation of partially missing diagnosis date.

Removed the restriction that summary statistics will be given by dose/frequency in section 9 and added details about what will be produced in appendices.

Clarified which variables are to be summarised at baseline and added that baseline tables are to be produced for all 3 populations in section 9.3.

Added medical history and concomitant medications appendix in section 9.4.

Clarified what tables and figures are produced for the primary analysis in section 10.1.

Added sentence to section 10.2.1 to allow comparison of each repeated measure secondary endpoint model with the null model including only an intercept term.

Clarified what tables and figures are produced for secondary analysis in section 10.2. Also added a McNemar's test for differences in antibody positivity, linear mixed effects models for 90-minute data and C-peptide correlation analyses. Added that secondary analyses will be carried on both "evaluable" and "analysis" population.

Clarified for which variables profiles will be produced and t-tests performed in section 11.1. Also added a number of follow-up summary tables equivalent to many of the tables produced at baseline.

Included details on what are expected ARs in section 11.4.

Added more detail on what AE tables will be produced in section 11.7.

Added sentence to section 12 stating that metabolic variable profiles will be split into fasting and non-fasting visits. Added that bar plots showing change from baseline in endpoints and 90-minute data may be produced.

Changed the precision of p-value reporting to 5 DPs in section 13.

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18 Appendix

DOSE AND FREQUENCY COMMITTEE CHARTER: DILFREQUENCY TRIAL Version 1.0, 09 January 2015