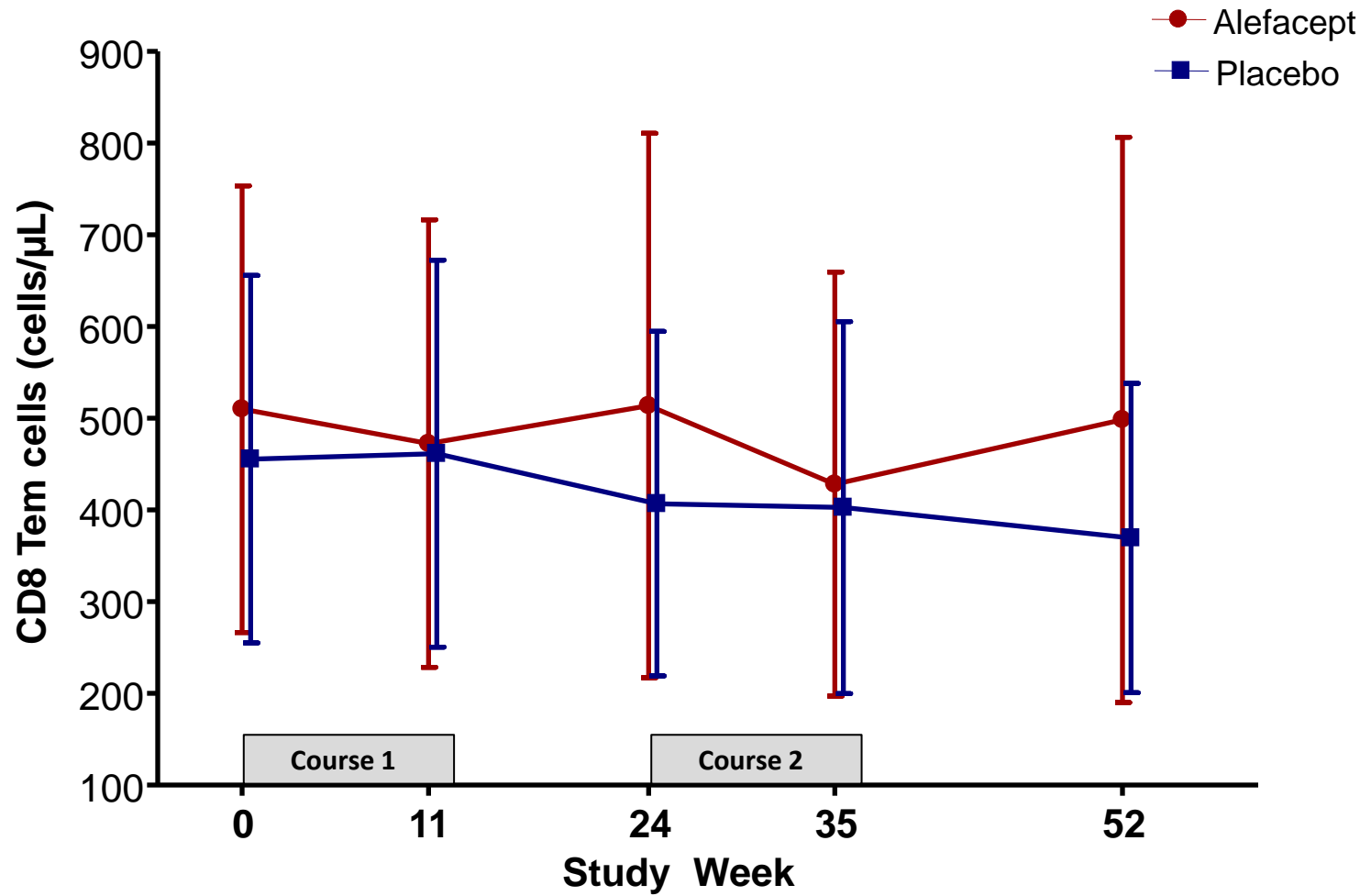


Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Rigby MR, DiMeglio LA, Rendell MS, et al. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol* 2013; published online Sept 23. [http://dx.doi.org/10.1016/10.1016/S2213-8587\(13\)70111-6](http://dx.doi.org/10.1016/10.1016/S2213-8587(13)70111-6).

Supplementary Figure 2I: Calculated absolute counts of CD8 Tems



Supplementary Table 1. Flow cytometry panels**Panel design for characterization of T cells**

Fluorophor	Marker	Manufacturer	Cat. No.	Clone
PerCP-eF710	CD2	eBio	46-0029-42	RPA-2.10
BV421	CD127	BL	351310	A019D5
V500	CD4	BD	560769	RPA-T4
BV650	CD8	BL	301041	RPA-T8
AF700	CD3	BD	557943	UCHT1
PE	CCR7	BL	353204	G043H7
PE-Cy7	CD45RA	BL	304126	HI100
PE-CF594	CD25	BD	562403	M-A251
AF488	Helios	BL	137223	22F6
AF647	FOXP3	BL	320114	206D

Panel design for characterization of B cells and other immune cell populations

Fluorophor	Marker	Manufacturer	Cat. No.	Clone
FITC	CD2	BD	347593	S5.2
PerCP-Cy5.5	CD123	BD	560094	7G3
V450	CD11c	BD	560369	B-ly6
V500	CD14	BD	561391	RPA-T4
BV605	CD27	BD	562655	L128
BV650	CD8	BL	301041	RPA-T8
AF647	CD16	BL	302020	3G8
AF700	HLA-DR	BL	307626	L243
PE	CD56	BL	318305	HCD56
PE-Cy7	CD19	BL	302215	HIB19
PE-CF594	CD3	BD	562280	UCHT1

Supplementary Table 2: Gating strategy for T and B cell populations

CD4, Non- Tregs	Live gate →singlets→lymph→CD3+→CD4+ →CD127+FOXP3neg	Naïve	CD45RA+CCR7+
		Central Memory	CD45RAnegCCR7+
		Effector Memory	CD45RAnegCCR7lo
Tregs	Live gate →singlets→lymph→CD3+→CD4+ →CD127neg/loFOXP3+		
CD8	Live gate →singlets→lymph→CD3+→CD8+	Naïve	CD45RA+CCR7+
		Central Memory	CD45RAnegCCR7+
		Effector Memory	CD45RAnegCCR7lo
B Cells	Live gate →singlets→lymph→CD3negCD16neg→CD19+		

Supplementary Statistical Methods

Computation of 2-hour C-peptide AUC: The 2-hour C-peptide AUC was calculated using the trapezoidal rule over a 2-hour period (0-120 minutes). The “time 0” C-peptide value was taken as the average of C-peptide values measured at time points -10 and 0 minutes. The AUC calculation was based on the available time points from the MMTT. Results reported as less than the lower limit of detection were imputed as half the lower limit of detection for that time point.

Imputation for missing Week 52 MMTT: The MMTT performed at the Week 52 visit was used, regardless of time out of window. For a participant who missed the entire Week 52 MMTT assessment, the missing AUC values were imputed using the following approach for the primary analysis of the primary endpoint: If the subject’s last observed AUC value was 0, the missing Week 52 AUC was imputed to zero. If the subject’s last observed AUC value was at time t and was >0 , then the missing AUC at Week 52 was imputed using data from subjects in the same arm who had available AUC values at both time t and Week 52. Specifically, a linear regression line and 90% confidence bands were fit where Week 52 AUC values were regressed on AUC values at time t (e.g. Week 24). In each arm, a missing Week 52 AUC value was imputed as the value predicted from the linear regression line.

Sensitivity analyses were also performed on the ITT population using the following approaches:

- Using observed data only (i.e. without imputing any missing Week 52 AUCs.)
- Optimistic: In the active therapy arm, a missing Week 52 AUC value was imputed as the value predicted from the estimated upper limit of a 90% confidence band about the linear regression line. In the control group, a missing Week 52 AUC value was imputed as the

value predicted from the estimated lower limit of a 90% confidence band about the regression line.

- **Conservative:** In the active therapy arm, a missing Week 52 AUC value was imputed as the value predicted from the estimated lower limit of a 90% confidence band about the linear regression line. In the control group, a missing Week 52 AUC value was imputed as the value predicted from the estimated upper limit of a 90% confidence band about the regression line.

Computation of 4-hour C-peptide AUC: The 4-hour C-peptide AUC was calculated using the same method as the 2-hour C-peptide AUC, only over a 4-hour time period (0-240 minutes). Missing 4-hour C-peptide AUC values were imputed using the same approach as the primary analysis of the primary endpoint.