SUPPLEMENTARY DATA

Supplementary Methods. Generation of DC products:

Monocytes from Leukopack were separated by elutriation in a closed ELUTRA^{TM} system and then cultured in a closed Aastrom Replicell System in the presence of IL-4 and GM-CSF to generate control DC. In parallel, the immunosuppressive DC (iDC) were generated identically however, sterile, QC-tested phosphorothioate-modified antisense oligonucleotides targeting the 5' end of the CD40, CD80 and CD86 gene primary transcripts were added to the DC in the Aastrom Replicell System at a final concentration of 3.3 micromolar. The sequences of each of the antisense oligonucleotides is: CD40: 5’ act ggg cgc ccg agc gag gcc tct gct gac 3’; CD80: 5’ ttg ctc acg tag aag acc ctc cca gtg atg 3’; CD86: 5’ aag gag tat ttg cga gct ccc cgt acc tcc 3’. On day 6 of the DC generation process, the cells were harvested and checked for viability and purity. The cells will be tested for viability, sterility (bacterial aerobic and anaerobic cultures and Gram stain), endotoxin level and mycoplasma as routinely performed by the IMCPL for cellular products used for human therapy at the UPCI. An aliquot of the freshly-generated DC were used for the first injection, while the remaining DC were aliquoted into sterile cryovials and cryopreserved for preparation of subsequent injections. Each injection was prepared as an adjustment of the cell number to the concentration of 2.5×10^6 cells in 1 mL of sterile PBS and divided among four 1mL tuberculin syringes for intradermal administration.

DC-Breg isolation:

Human B220+ CD19+ IL-10+ cells were isolated from buffy coats. After Ficoll separation from red blood cells, PBMC were selected along CD19 positivity using the human CD19 Microbeads kit (Millyteni Biotec) in magnetic MACS LS separation columns (1.5×10^9 PBMC per column). These cells were stimulated overnight with an agonistic CD40 antibody before being labeled with the human IL-10 secretion assay detection kit (Milltenyi Biotec; the kit detects surface-IL-10 and retains the cells alive for functional studies) and co-stained with human CD11c and B220 antibody. Cells were then flow-sorted into B220+ CD11c- IL-10+ cells and used for suppression studies or further characterised.

DC-Breg Suppression Assay:

To determine if the B220+ CD11c- B-cells represented, or contained one or more functionally-suppressive populations, T-cell proliferation *in vitro* in allogeneic mixed leukocyte reactions (MLR) was determined in the presence or absence of the parental B220+ CD11c- population. Although the B220+ CD11c- population was not suppressive *per se*, a relatively-abundant subpopulation of B220+ CD19+ CD11c- IL-10+ cells was (Online Supplemental Figure S3a, S3b). Unlike the other currently-characterized mouse and human suppressive B-cells (also termed B regulatory cells or Bregs) {Yanaba, 2008 #939; Iwata, 2010 #1003}, IL-10 was not responsible for the suppressive effect of our putative Bregs as these cells were still able to suppress proliferation of T-cells in allogeneic MLR in the presence of a neutralizing IL-10 antibody (Online Supplemental Figure S3a, S3b). The human suppression co-culture supernatants were analyzed for cytokine production and, as shown in Online Supplemental Figure S3b, a statistically-significant suppression of pro-inflammatory TNFalpha, IFNgamma and IL-2 was observed in co-cultures with our putative Bregs added.

SUPPLEMENTARY DATA

Supplementary Table 1. Inclusion and Exclusion Criteria. All patients enrolled were on insulin replacement therapy.

Inclusion criteria:		
Written informed consent conforming to the institutional guidelines obtained from the patient.		
Documented evidence of insulin-requiring type 1 diabetes of >5 years duration.		
Adequate immune competence (reactivity to CEF viral peptide pool)		
Age 18-60.		
Adequate hematologic function:	Absolute neutrophil count > 1,000/mm^3	
	Absolute lymphocyte count > 1,000/mm^3	
Hemoglobin > 9 gm/dl		
Platelets > 100,000/mm3		
Liver function tests:	-Bilirubin (total) < 1.7 mg/dl	
	-Alkaline phosphatase < 78 u/L (2 x ULN)	
	-SGOT < 54 u/L (2 x ULN)	
	-Lactic dehydrogenase < 180 u/L (2 x ULN)	
i) Kidney profile:	-Serum electrolytes	Sodium 135-145 mEq/L
		Potassium 3.5-5.0 mEq/L
		Bicarbonate 21-28 mEq/L
		Chloride 100-108 mmol/L
		Serum creatinine <4.5 mg/dL (3 x ULN)
		BUN 8-25 mg/dL
j) No prior history of radiation therapy, immunotherapy, or chemotherapy		
absence of HIV, HSV, HBV, HCV viral infections		
Exclusion criteria:		
One or more of the Eligibility Criteria are not met.		
A significant history or current evidence of cardiac disease including, but not limited to, congestive heart failure, coronary artery disease, angina pectoris, uncontrolled hypertension, serious arrhythmias; or myocardial infarction within the previous six months.		
Evidence of active infection requiring antibiotic therapy.		
History of other concurrent diseases.		

Pregnant or lactating women.
Patients requiring systemic corticosteroids (except when administered in life-threatening circumstances)
Any other immune disorder including but not limited to other autoimmune diseases like rheumatoid arthritis, systemic lupus erythematous, multiple sclerosis, or ankylosing spondylitis
pregnancy
history of radiation therapy, immunotherapy, or chemotherapy
breastfeeding
other particle or cell vaccine therapies

Supplementary Table 2. Specific immune cell populations measured in peripheral blood of patients by flow cytometry

T-cells:
CD3+ CD8+
CD3+ CD4+
CD4+ CD45RA+ (naive)
CD8+ CD45RA+ (naive)
CD4+ CD69+ (activated)
CD8+ CD69+: (activated)
CD3+ CD8+
B-cells:
B220+ CD11c-
Monocytes/dendritic cells:
CD83+ HLA-DR+ inside CD11c gate (myeloid DC)
Regulatory cells:
FOXP3+ $CD25^{HIGH}$ inside CD4 gate (CD4+ Foxp3+ Tregs)
B220+ CD11c- CD19+ IL-10+ (DC-Bregs)

SUPPLEMENTARY DATA

Supplementary Table 3. Antibodies and isotypes used to characterise and measure the B-cell markers as well as to flow sort the B-cells to DC-Bregs

Marker	**Clone**	**Company**
Mouse IgG1 isotype	MOPC-21	BD Biosciences
CD5	UCHT2	BD Biosciences
CD38	HIT2	BD Biosciences
Rat IgG2a isotype	R35-95	BD Biosciences
B220	RA3-6B2	BD Biosciences
CD1d	CD1d142	BD Biosciences
CD24	ML5	BD Biosciences
CD11c	B-ly6	BD Biosciences
CD19	SJ25C1	BD Biosciences
CD40	5C3	BD Biosciences
IgD	IA6-2	BD Biosciences
IgM	G20-127	BD Biosciences
CD21	B-ly4	BD Biosciences
CD10	HI10a	BD Biosciences
CD27	M-T271	BD Biosciences
KI67	B56	BD Biosciences
CD3	HIT3a	BD Biosciences
CD4	RPA-T4	BD Biosciences
CD8	RPA-T8	BD Biosciences
CD44	G44-26	BD Biosciences
CD83	HB15e	BD Biosciences
CD45RA	HI100	BD Biosciences
CD69	FN50	BD Biosciences
IL-10 (Internal)	JES3-19F1	BD Biosciences
Tregs Staining Kit (Foxp3)	PCH101	eBioscience

SUPPLEMENTARY DATA

Cell staining with antibodies for flow cytometry:
Freshly-isolated patient blood was diluted 1:1:1 with sterile 1x PBS and Ficoll and then layered on the bottom of a sterile polypropylene tube. The blood was then centrifuged at 1600 rpm for 30 minutes and the PBMC layer was removed. The PBMC were further washed in 1x PBS. 1 x 10^5 PBMC were stained with the antibodies listed above as appropriate and parallel cells were stained with isotype controls. FACS analysis was performed within 24 hours. For internal IL-10 or Foxp3 detection, we used a commercially-available cell permeabilisation kit (eBioscience) according to the manufacturer's directions.

Supplementary Table 4. Laboratory Measurements

Biochemistry (blood)	***Biochemistry (urine)***	***Hematology***
Albumin	***Macroscopic-***	Activated PTT
Alkaline phosphatase	Urine Color, Character, Volume, Specific gravity	Prothrombin time
Alanine aminotransferase	pH	Complete blood count
Aspartate aminotransferase	Protein	WBC
Insulin C-peptide	Glucose	RBC
Creatinine	Ketones	Hgb
Glucose	Bilirubin	HCT
Glycosylated HbA1c	Blood	MCV
Insulin	Urobilinogen	MCH
LDH	Nitrite	MCHC
LDL	Leukocyte esterase	RDW
VLDL		Mean platelet volume
Cholesterol	***Microscopic-***	Automated differential
Triglyceride	Urine WBC, RBC	Poly
HDL	Bacteria	ABS poly
Na	Squamous epithelial cells	Lymphocytes
K		ABS lymphocytes
Cl		Monocytes
CO_2		ABS Monocytes
Total bilirubin		Eosinophils
Thyroglobulin		ABS Eosinophils
Total protein		Basophils
TSH		ABS Basophils
Urea nitrogen		Platelets
		Reticulocytes
		ABS Reticulocytes

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Immune monitoring

Cytokines and concentration in serum	LincoPLEX, Beadlyte kits	IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, TGF-beta, TNFalpha, MCP-1, IFNgamma
Autoantibodies	Kronus Inc. kits	insulin, IA-2, GAD65
		a) Anti-nuclear antibody (with titer)
		b) Thyroglobulin antibody (with titer).
Cellular proliferation (mixed leukocyte culture)	BrdU Flow Kit, BD Biosciences	BrdU incorporation by FACS; BrdU Flow Kit, BD Biosciences
ELISPOT	CEF Peptide Pool, Mabtech	IFNgamma production (in response to viral peptides)
FACS		measurement of cells listed in Table T2

Supplementary Table 5. TABLE ACCOMPANYING ONLINE Supplementary Figure 7. Characterisation of B-cells upregulated in frequency in response to DC administration. The B220+ CD11c- B-cells comprise on average 1.0 - 3.3% of total PBMC in normal human individuals. Based on FACS analysis, the Table shows the relative levels of surface markers and the % of the cells in the B220+ CD11c- and total PBMC population that exhibit the characteristics shown in each column.

Marker	Levels	% of B220+ CD11c- population	% total PBMC
CD24	high	88.6	3.2
CD38	low	7.5	0.3
CD21	Absent/low	4.3	0.1
CD8	low	9.5	3.1
IgD	Absent/low	4.0	0.9
IgM	Absent/low	22.5	0.9
CD1d	Low/intermediate	16.9	0.7
CD5	high	81.8	3.6
CD40	low	20	0.6
CD10	Low/intermediate	5.2	1.2
CD27	high	35	9.3

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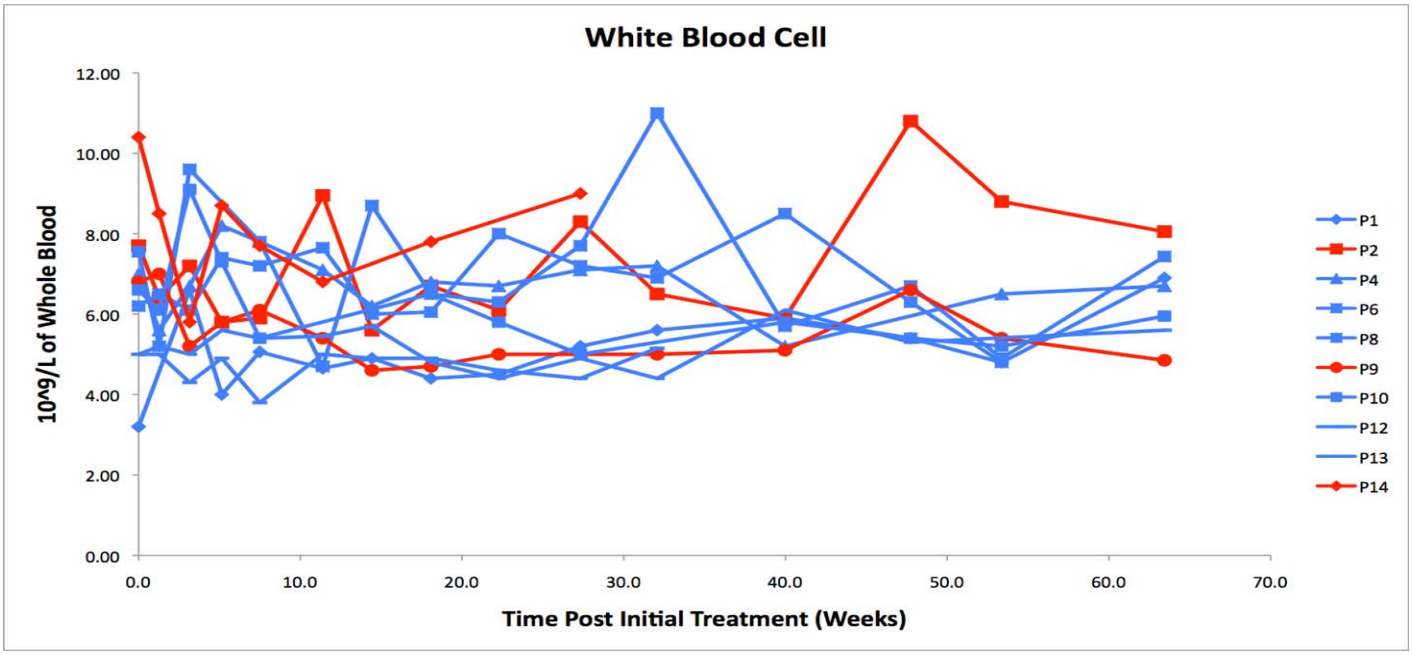
IL-10	high	83.8	16.0
CD38+ CD24+		6.3	0.2
CD38+ $CD24^{LOW/-}$		0	0
$CD38^{LOW/-}$ CD24+		83.1	3.0
CD38- CD24-		10.5	0.4
CD5+ CD1d+		12.5	0.5
CD5+ CD1d-		68.1	3.0

Supplementary Figure 1. Blood cell frequencies with reference range included as dashed lines (upper and lower limits of physiological ranges). The graphs show the numbers of each of the listed cell populations ascertained by standard hematology methods during each week of the trial. Week 0 is considered baseline, pre-treatment:

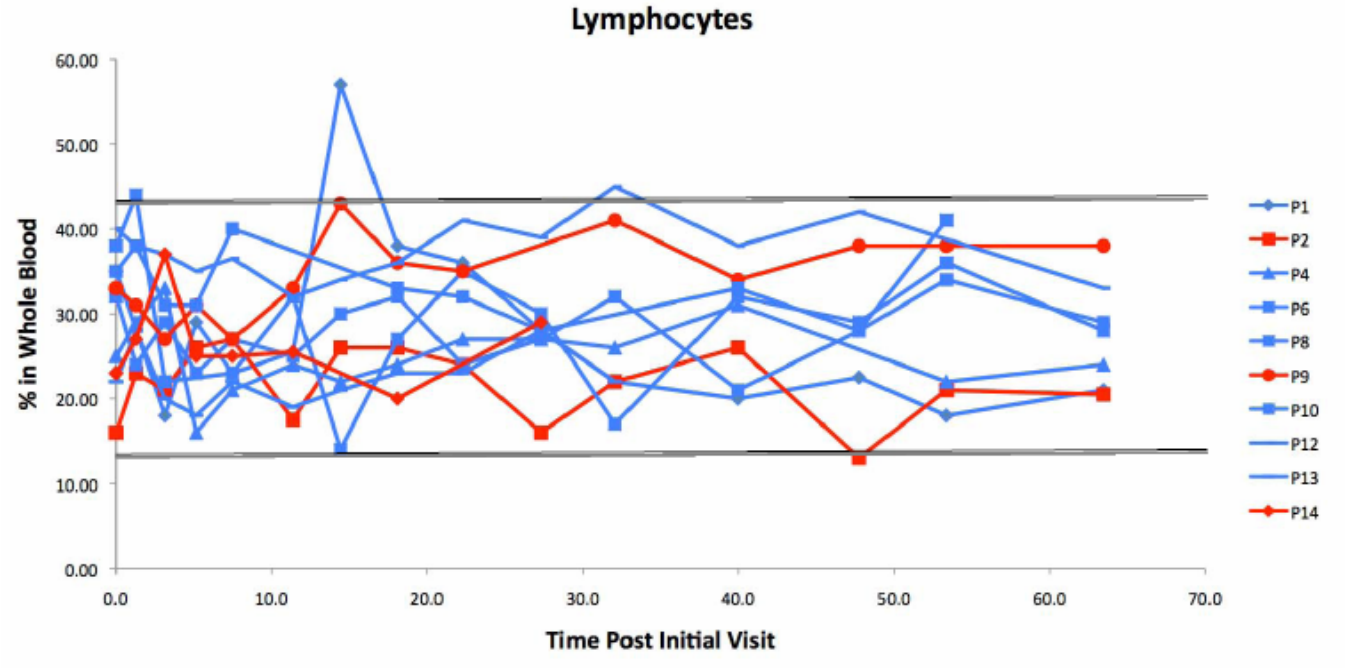
WBC
RBC
Poly
ABS poly
Lymphocytes
ABS lymphocytes
Monocytes
ABS Monocytes
Eosinophils
ABS Eosinophils
Basophils
ABS Basophils
Platelets
Reticulocytes
ABS Reticulocytes

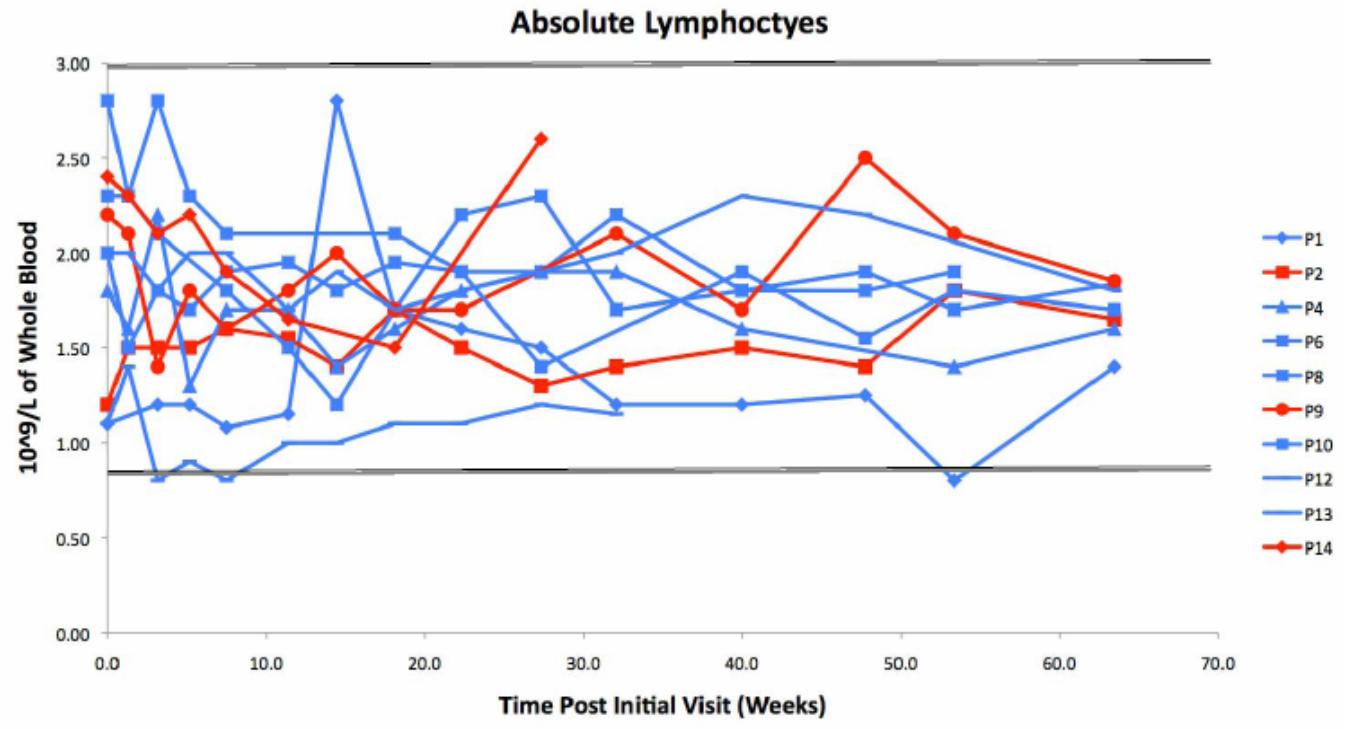
The standard range of each of the cell populations is indicated by dashed horizontal lines in the graphs which show the highest and lowest number that is considered physiologic. These reference ranges are those in use by the Hematology lab of the UPMC-CTRC. The blue-coloured symbols represent the iDC recipients and those in red the control DC recipients. The legend to the right indicates the symbol associated with each individual patient.



SUPPLEMENTARY DATA

Supplementary Figure 1 (cont'd)





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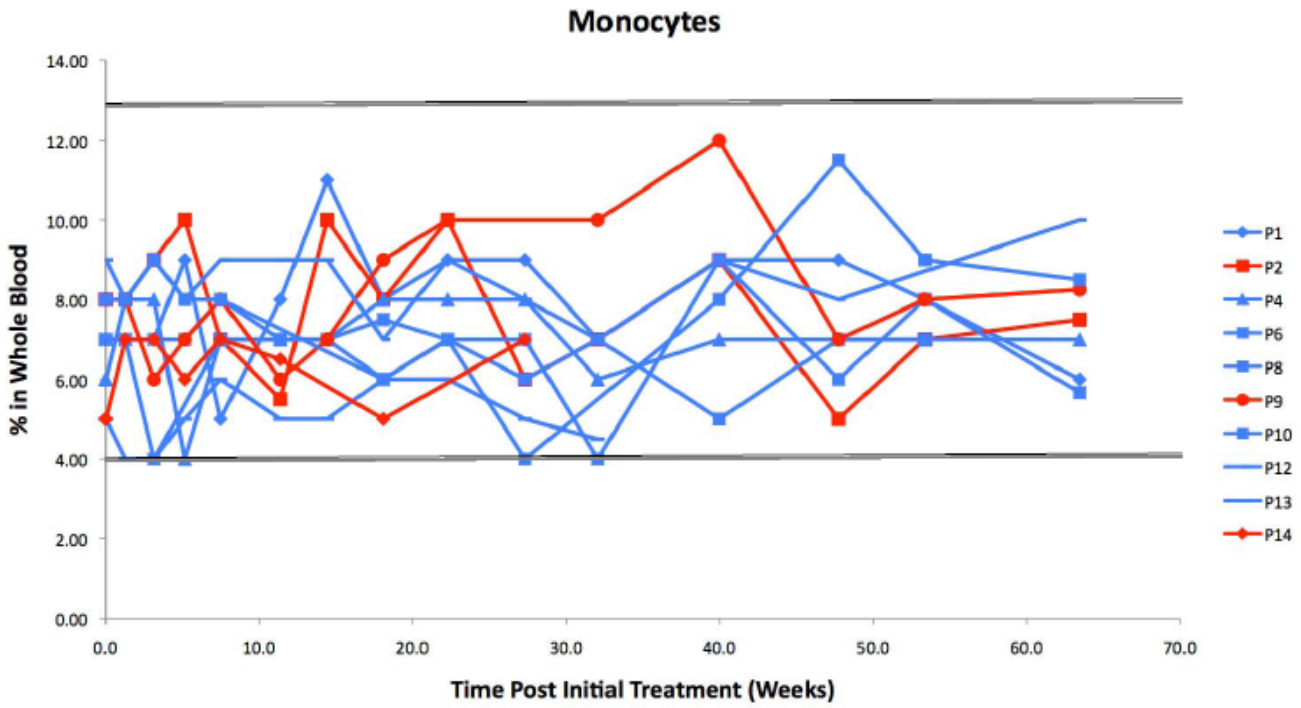

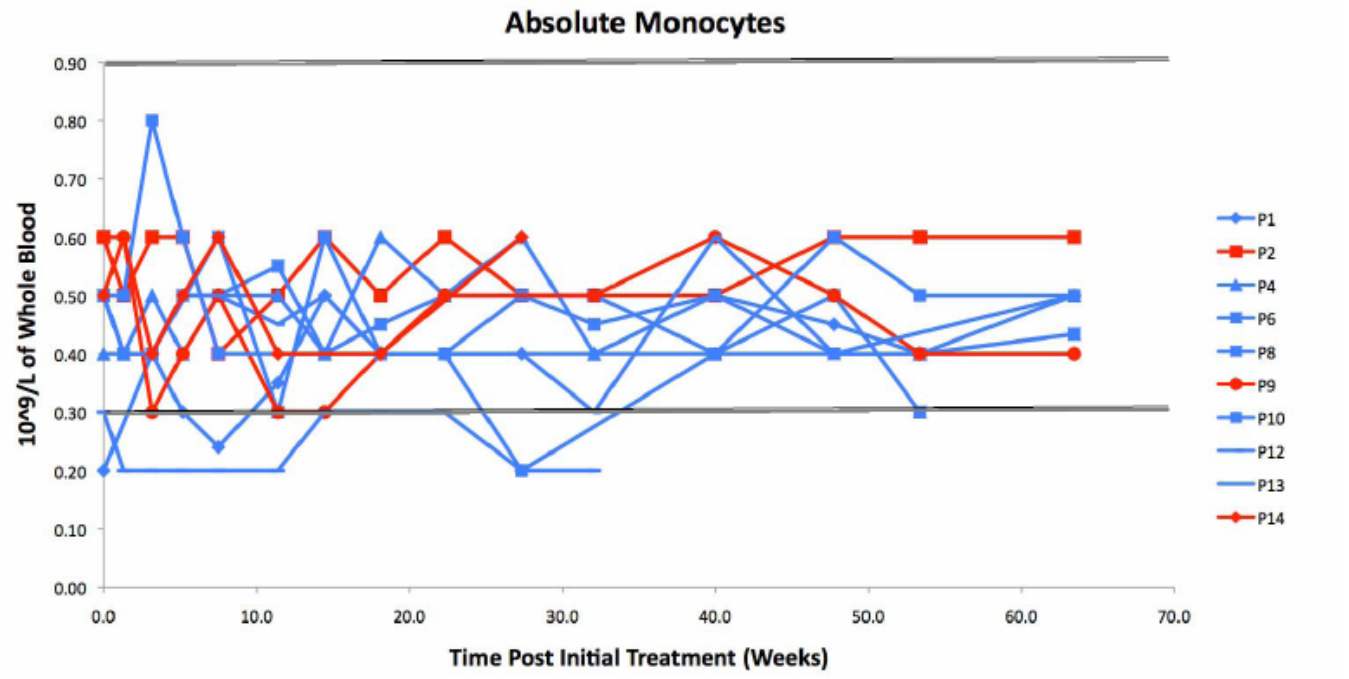

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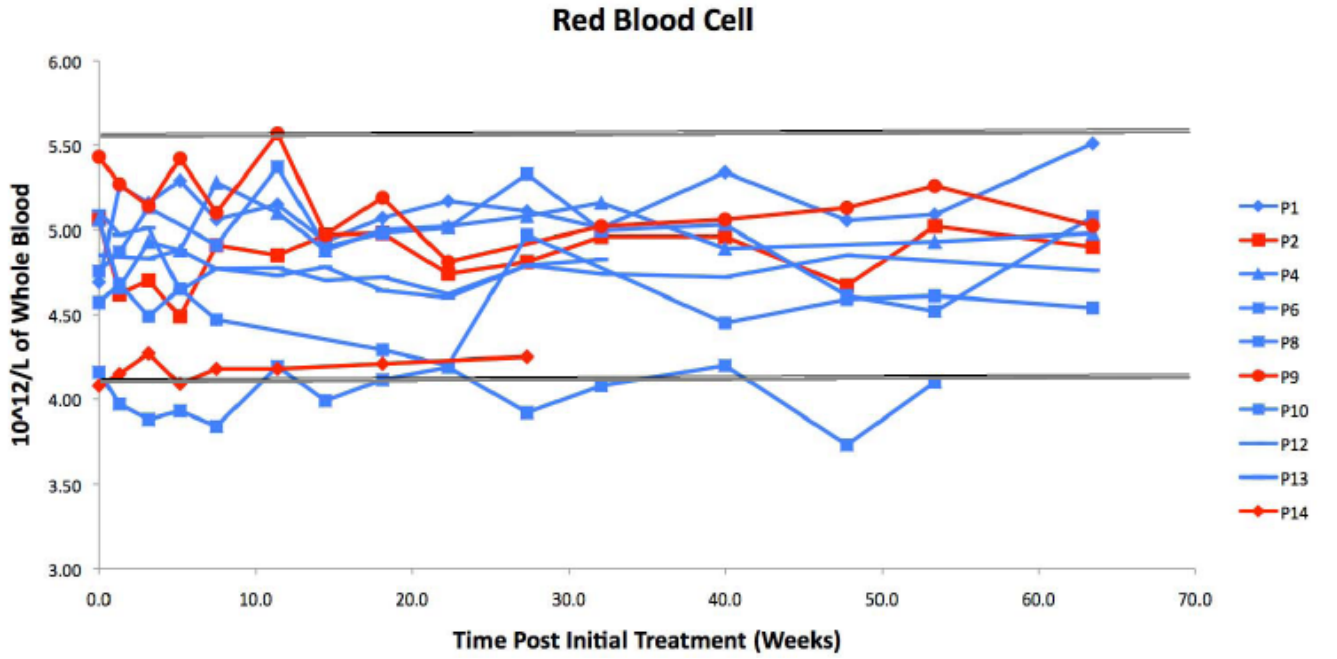

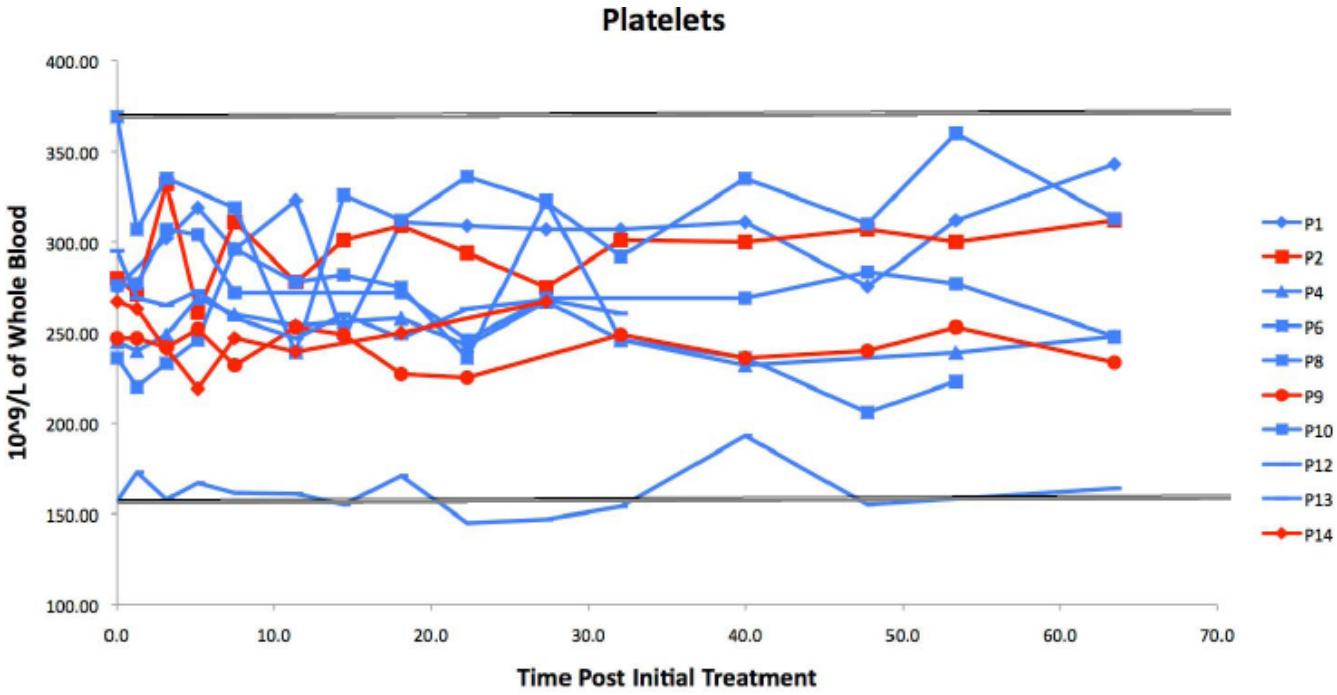

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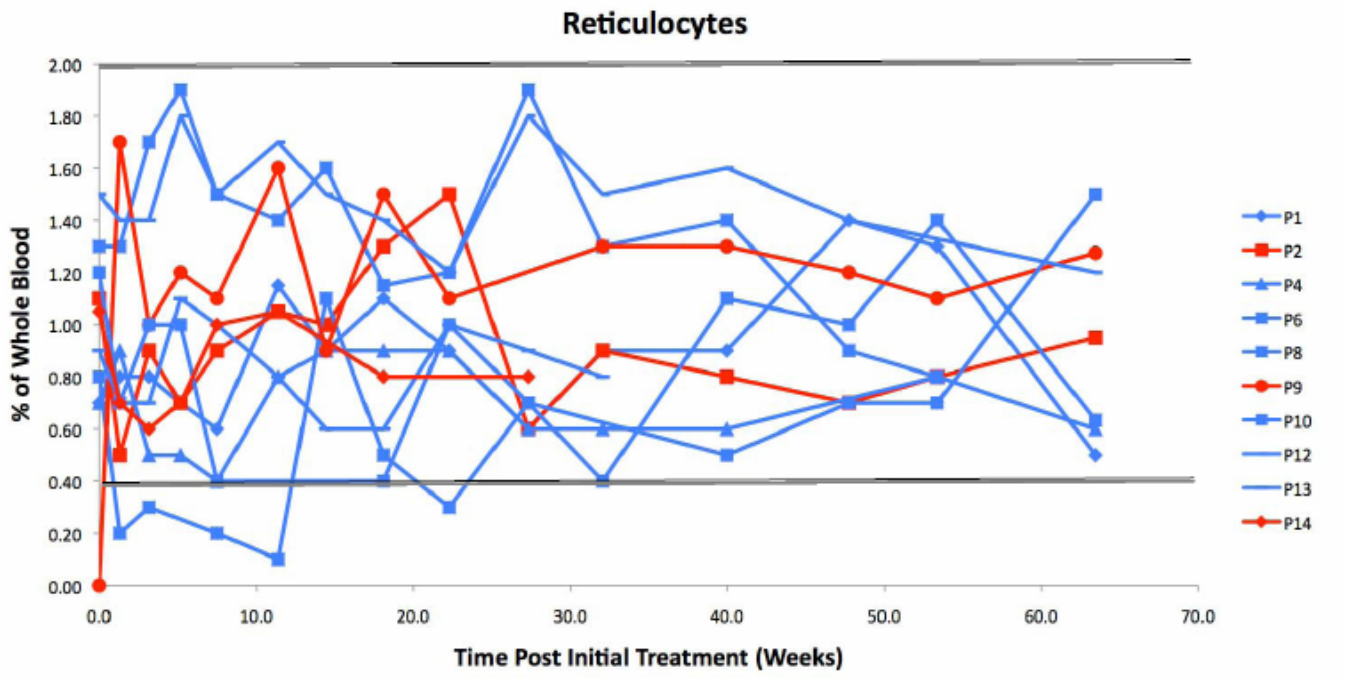

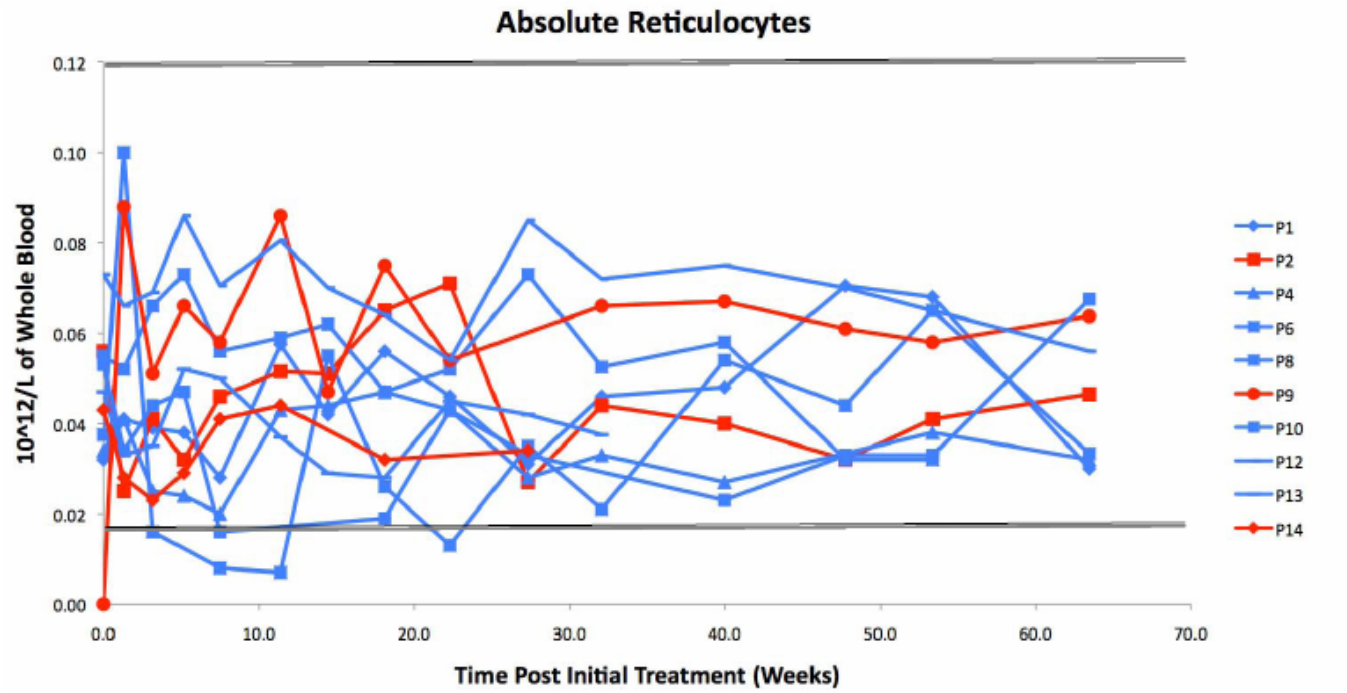

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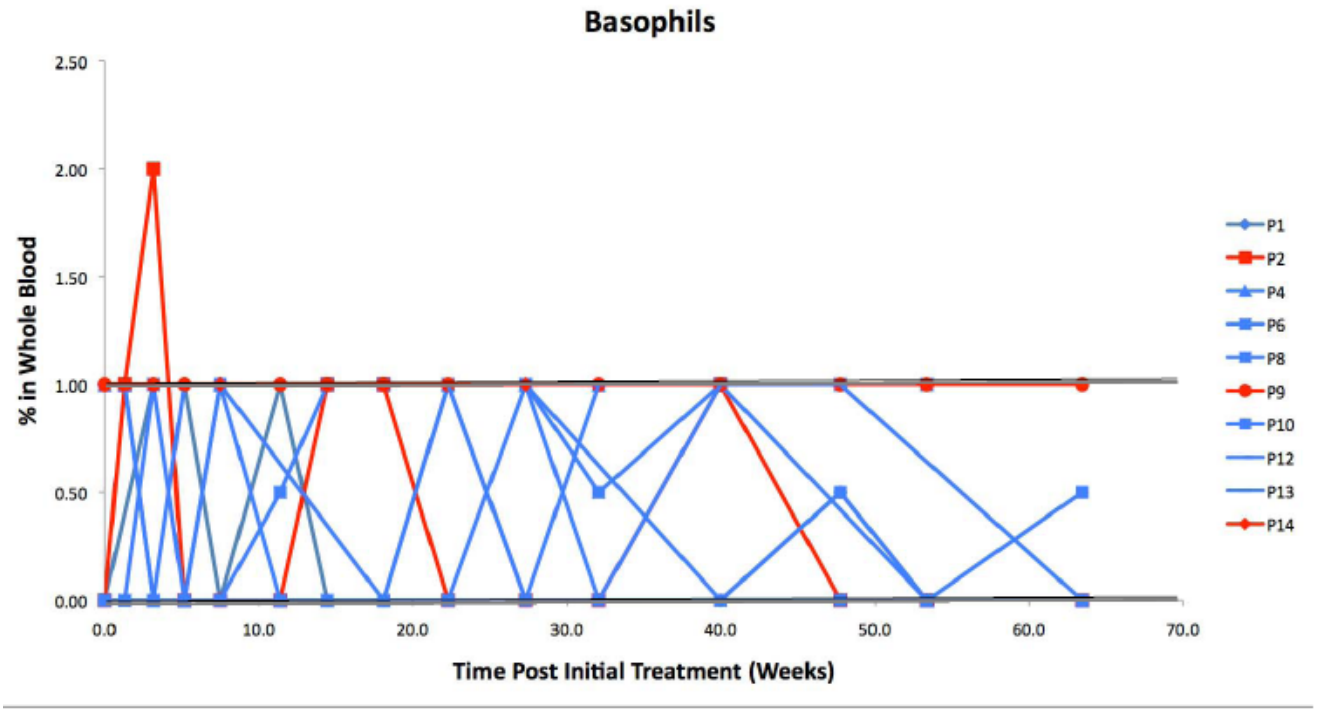

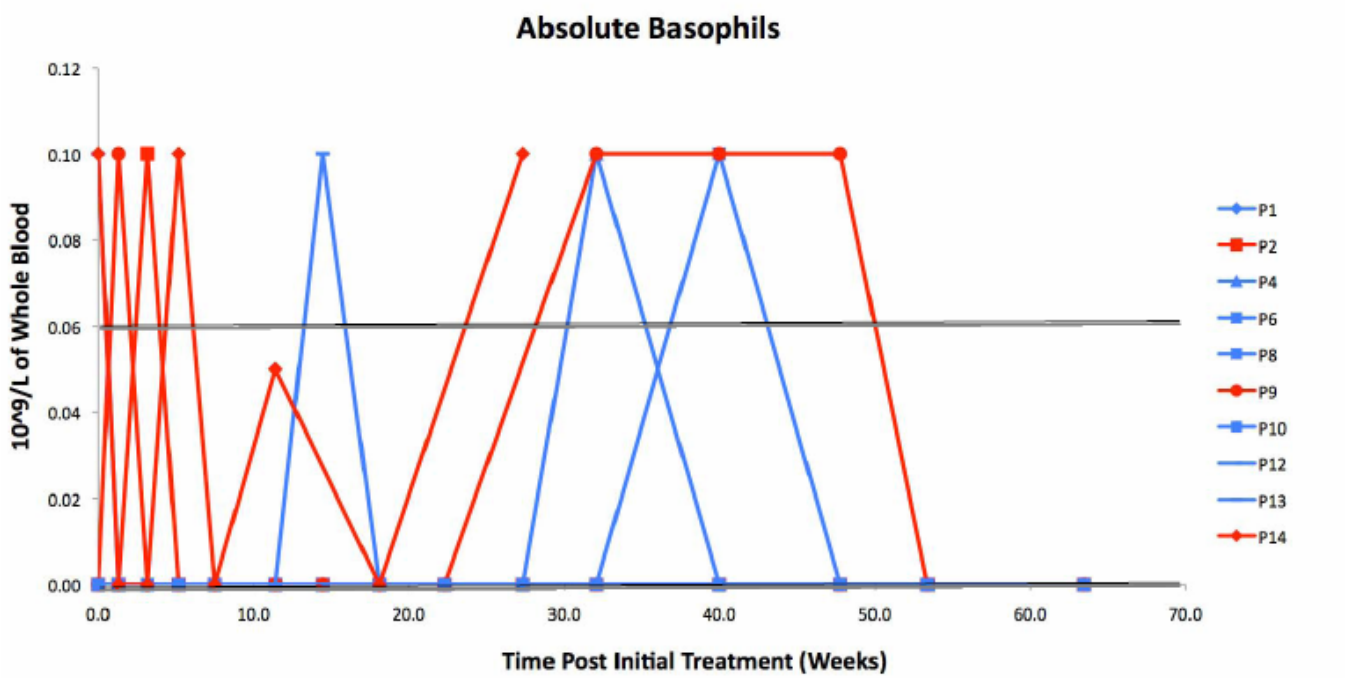

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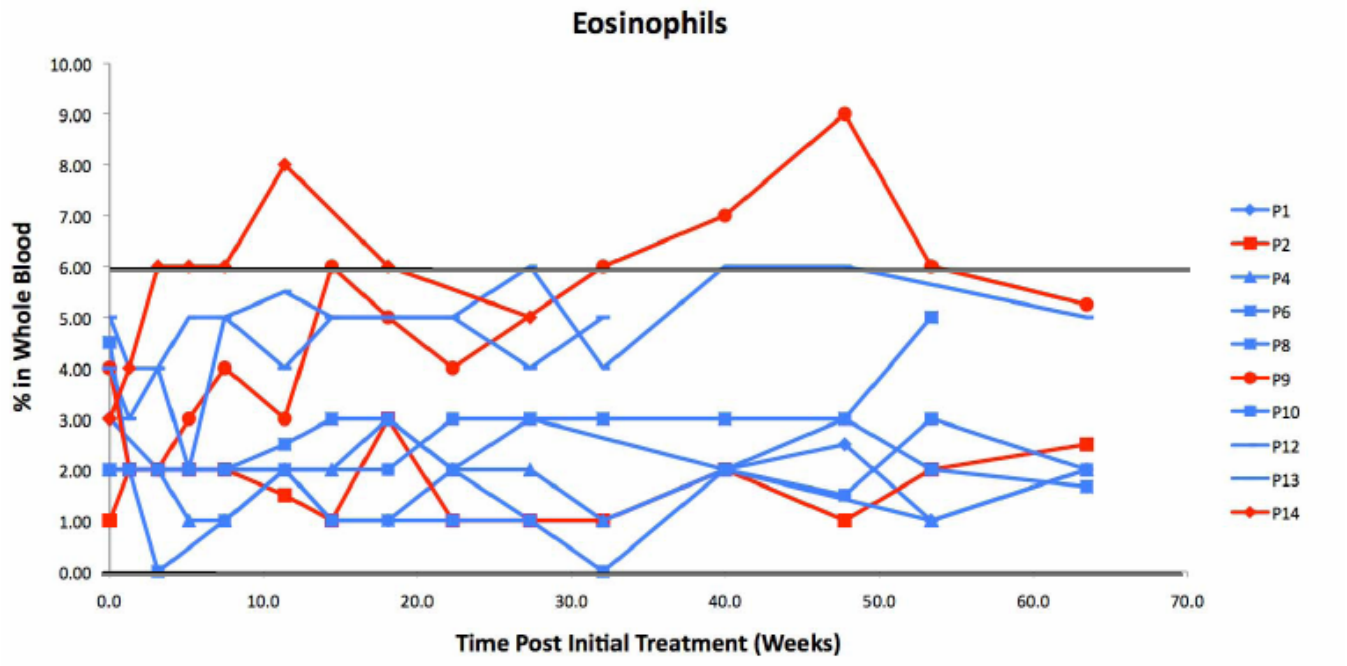

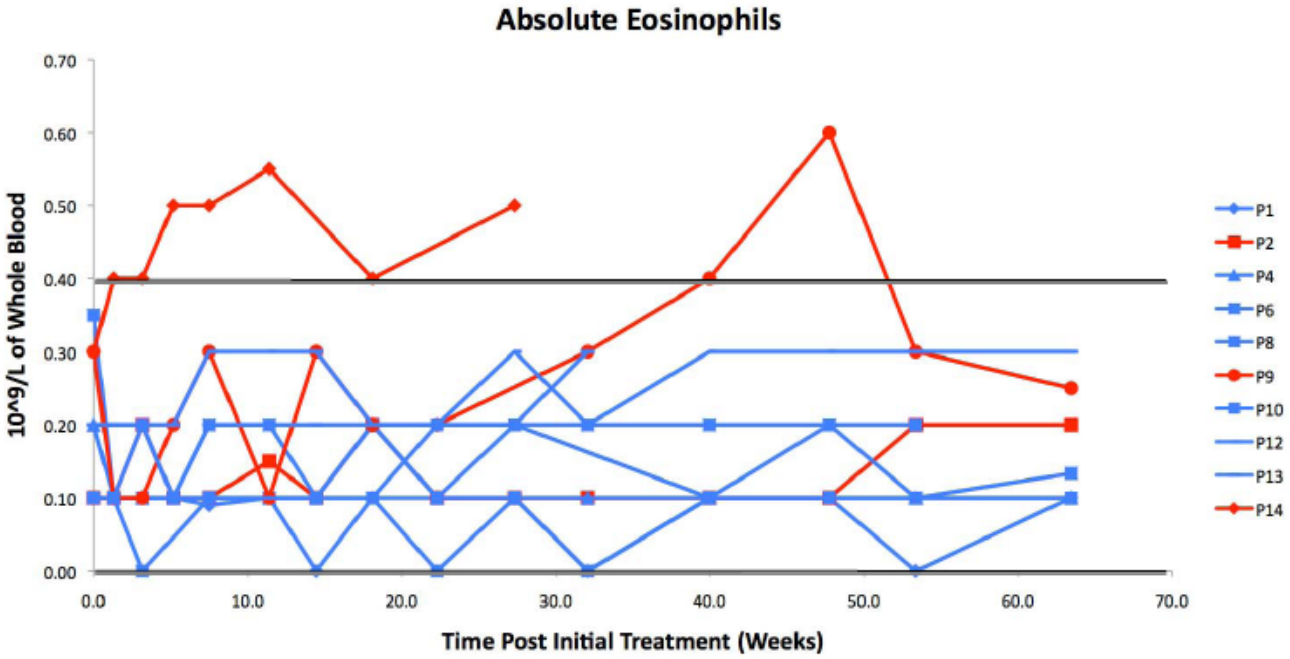

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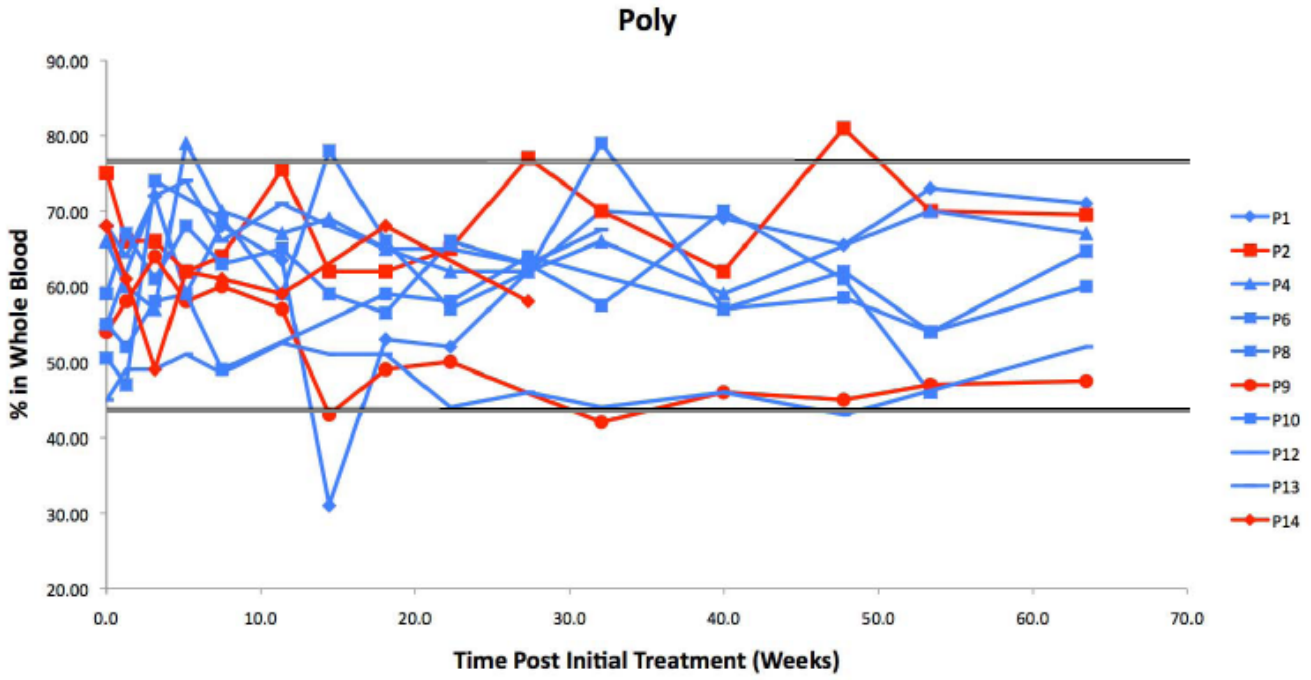
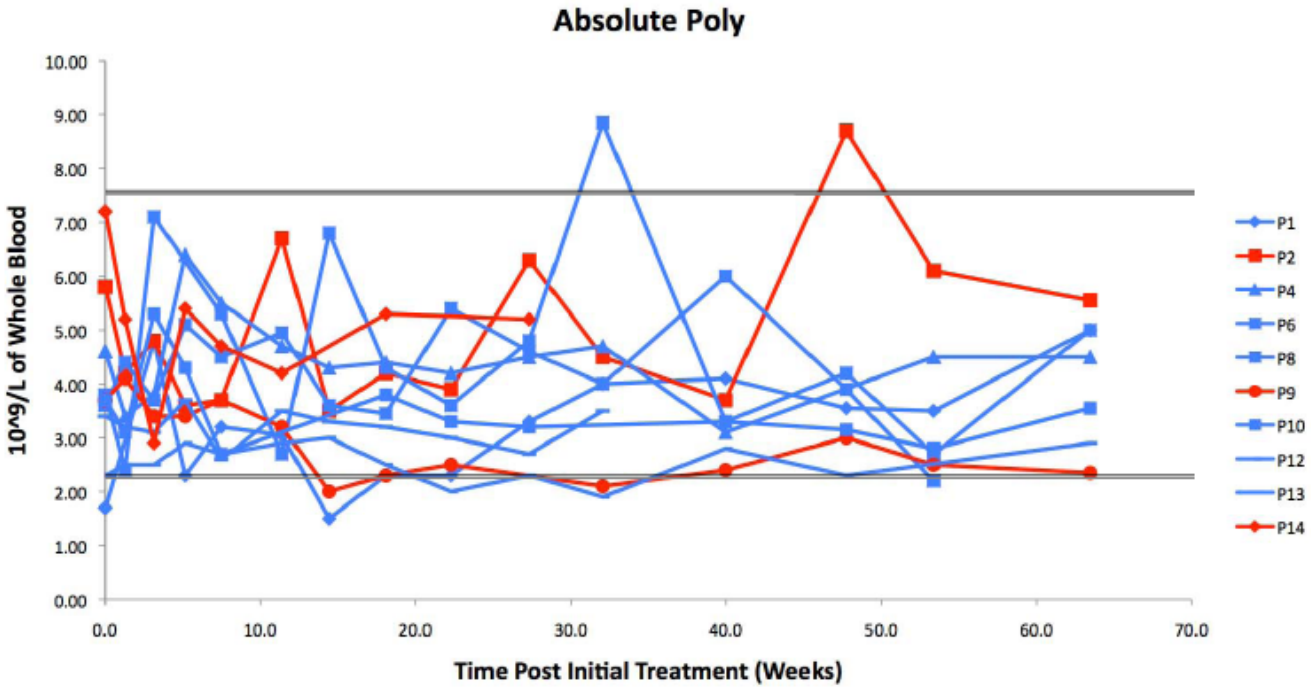
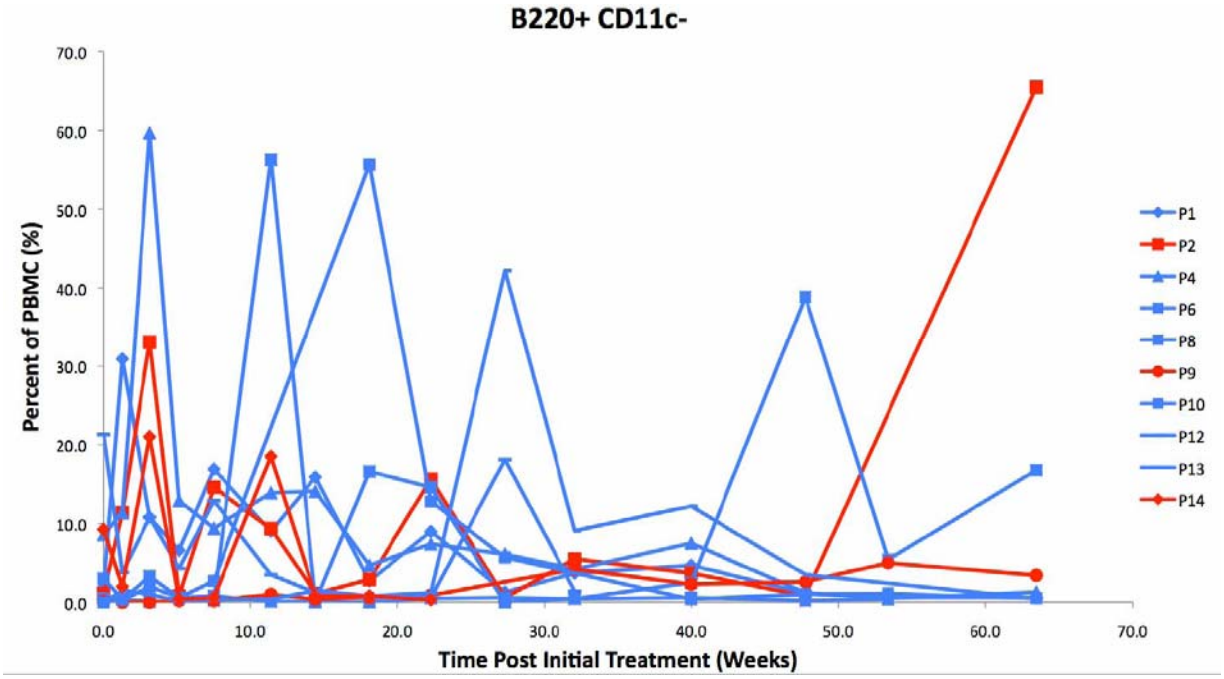




SUPPLEMENTARY DATA

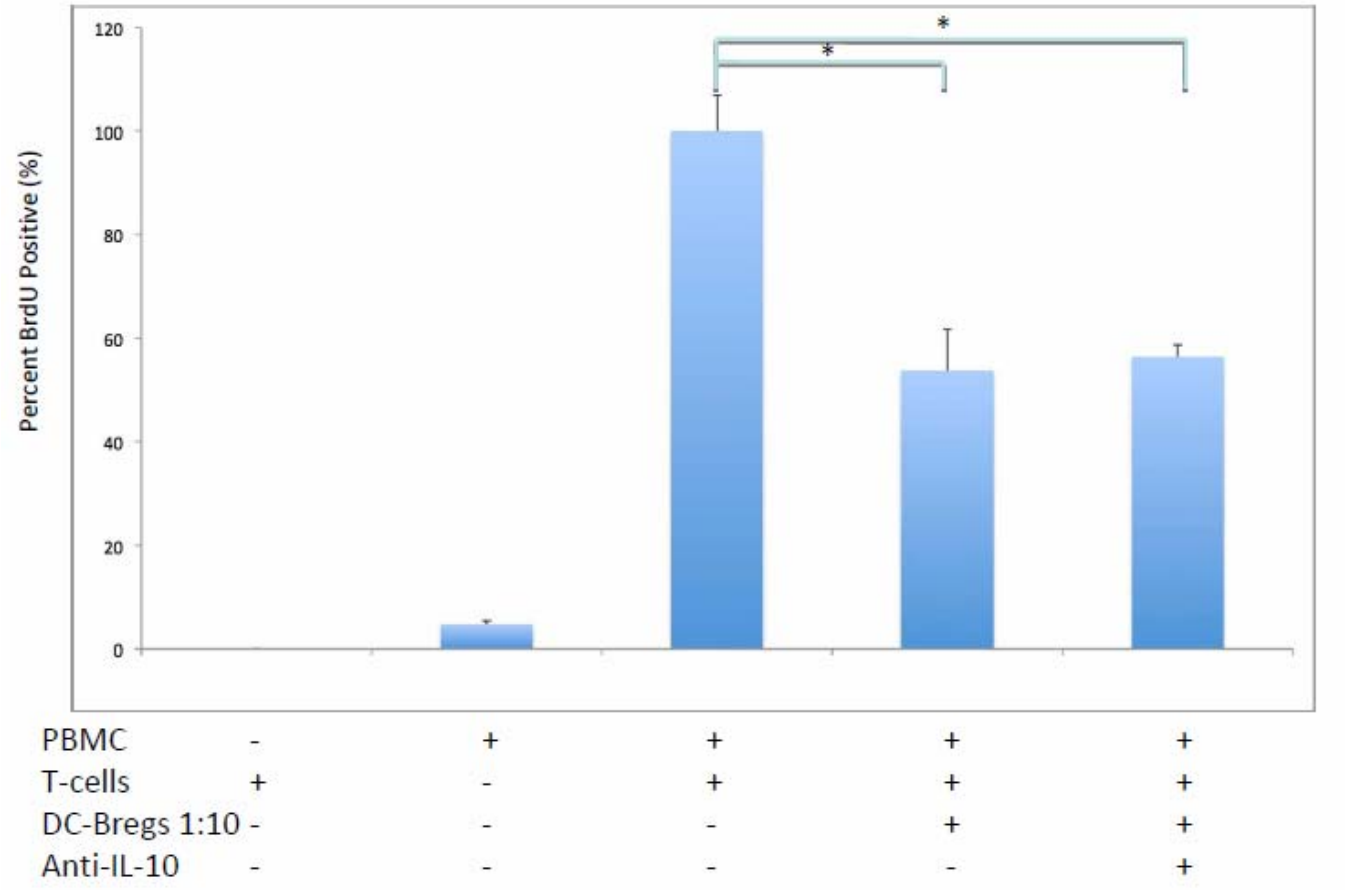
Supplementary Figure 2. Intra-abdominal intradermal DC administration increases the frequency of B220+ CD11c- B-cells in the PBMC of T1D patients. The frequency of B-cells in PBMC was measured by FACS at baseline and at each of the weeks of the trial shown in the x-axis. The graphs show the frequency of B220+ CD11c- cells in PBMC at each of the weeks of the trial. Week 0 corresponds to baseline, pre-treatment level. The symbols and lines in blue represent the iDC recipients and those in red represent the cDC recipients. The legend to the right of each graph shows the symbols that correspond to each individual patient (P). The values were measured in freshly-obtained blood at each of the weeks of the trial, shown on the x-axis in the graph.



SUPPLEMENTARY DATA

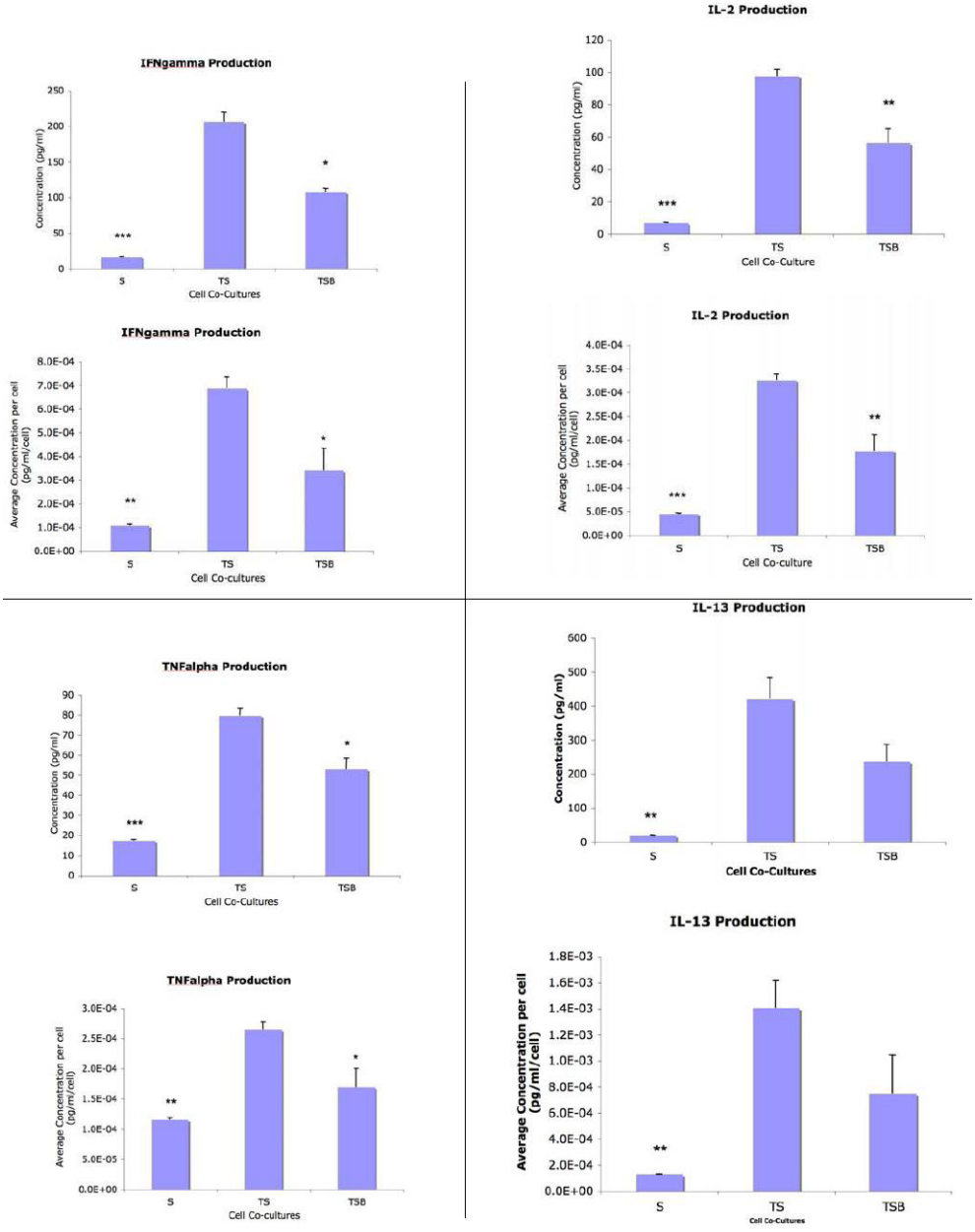
Supplementary Figure 3.

a) A subpopulation of B220+ CD19+ CD11c- IL-10+ B-cells inside the DC-expanded B220+ CD11c- population exhibits suppressive activity *in vitro* in allogeneic mixed leukocyte reaction (MLR) even in the presence of a neutralizing IL-10 antibody. Standard one-way MLR was conducted in vitro in co-cultures of T-cells enriched from PBMC of the DC-treated volunteers and irradiated allogeneic PBMC. As shown in the matrix below the graph, the MLR was conducted in the presence or absence of an equivalent number of patient-derived flow-sorted DC-Bregs as well as in the presence or absence of a human-specific neutralizing IL-10 monoclonal antibody (10 micrograms/mL). The bars represent the mean proliferation percentage of the patients' T-cells where the co-cultures in the absence of DC-Bregs are taken to represent 100% proliferation. The asterisks at the top of the graph identify the co-cultures where the differences in T-cell proliferation were statistically-significant ($p<0.05$, two tailed ANOVA, n=4)



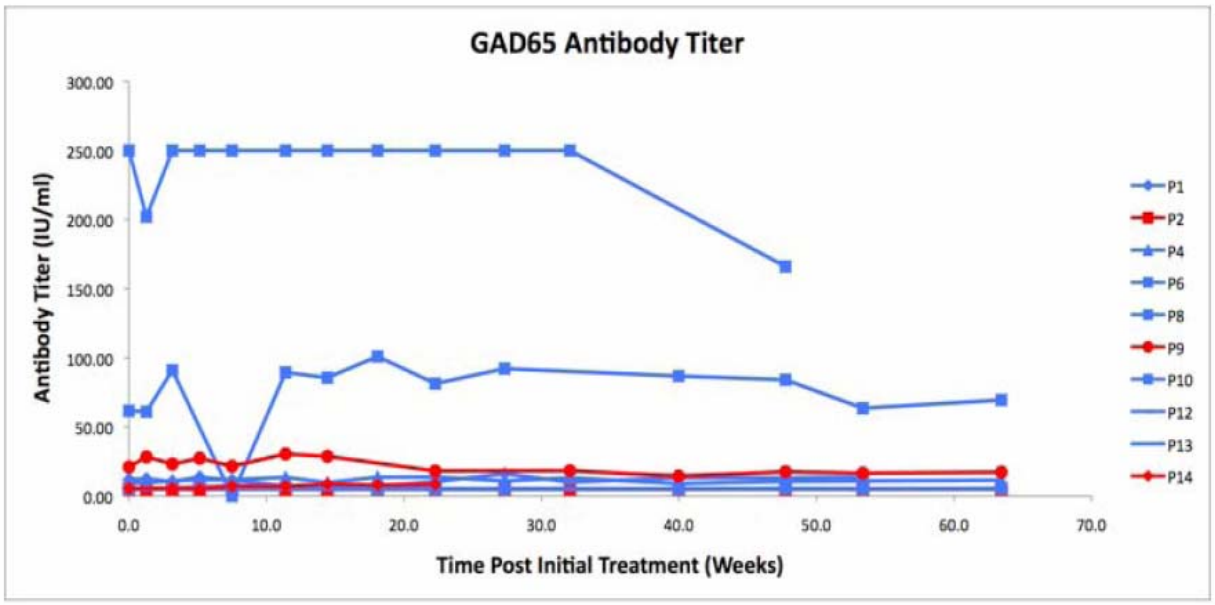
b) DC-Breg suppression *in vitro* in MLR is associated with impaired production of TH1-type pro-inflammatory cytokines. Supernatants from the day 5 suppression co-cultures (Figure 3b) were collected and probed for the presence and concentrations of pro- and anti-inflammatory cytokines using Luminex Multianalyte technology (LincoPLEX, Beadlyte). The only detectable cytokines whose concentrations were physiologically-relevant were TNFalpha, IFNgamma, IL-2 and IL-13. For each set of two graphs, the one at the top shows the concentrations of the cytokines in the supernatants while the one at the bottom shows the concentrations corrected by the total number of cells in the co-cultures. The data are shown for cytokine concentrations in triplicate co-cultures. The bars represent the means, the error bars the SEM and the

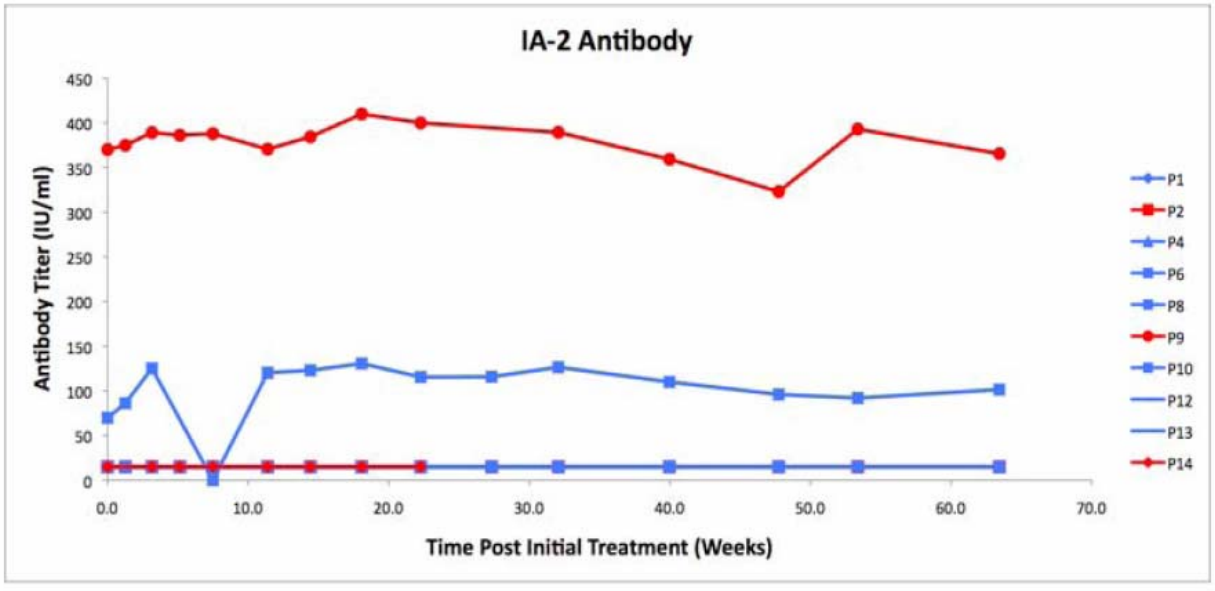
asterisks denote the comparator groups for statistical considerations. S indicates supernatants from cultures with irradiated stimulator PBMC alone; T indicates supernatants from cultures with patient T-cells alone; TS indicates supernatants from allogeneic stimulator PBMC:T-cell co-cultures; and TSB indicates the supernatants from allogeneic stimulator PBMC: patient T-cell co-cultures with patient PBMC-derived DC-Bregs added at a ratio of 1:10 (Bregs:T-cells). Differences in the cytokine concentrations between co-cultures of T-cells and allogeneic PBMC stimulators and those with added DC-Bregs were statistically-significant for IFNgamma, TNFalpha, and IL-2 (* indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$, ANOVA). The difference in IL-13 levels between stimulated and DC-Breg-supplemented cultures did not reach statistical significance ($p>0.05$, ANOVA).



SUPPLEMENTARY DATA

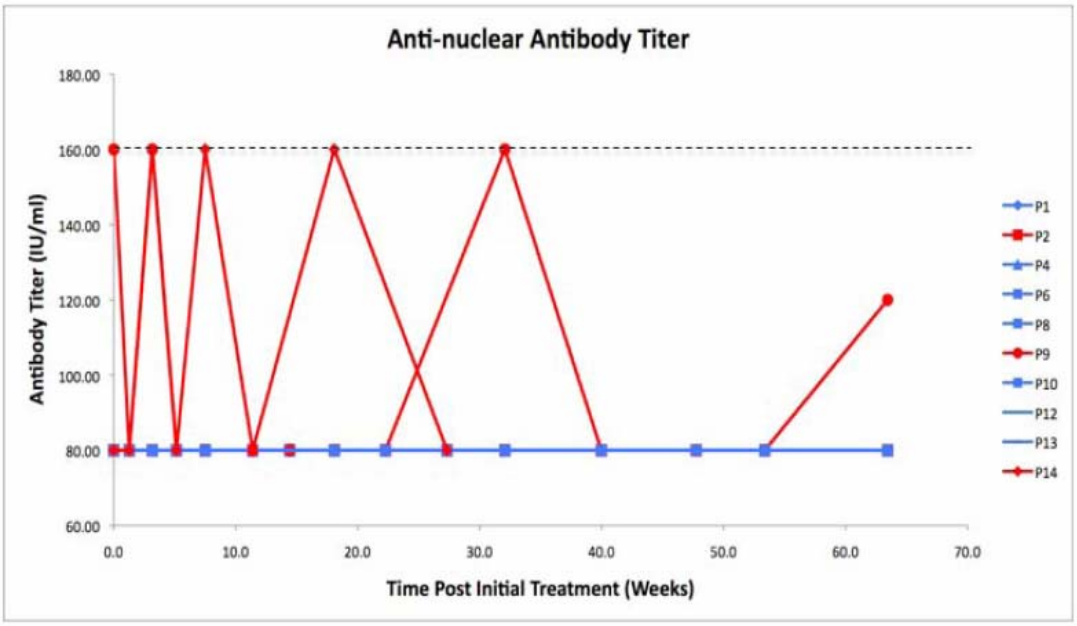
Supplementary Figure 4. Effects of cDC and iDC on serum diabetes autoantibody concentration. IA-2 and GAD65-specific antibody detection was carried out using the Kronus commercially-availablity kits as per the manufacturer's instructions using thawed frozen serum samples obtained from each patient at each of the weeks of the trial (refer to graph). Week 0 was baseline, pre-treatment. The blue-coloured symbols represent the iDC recipients and those in red the cDC recipients. The legend to the right indicates the symbol associated with each individual patient.

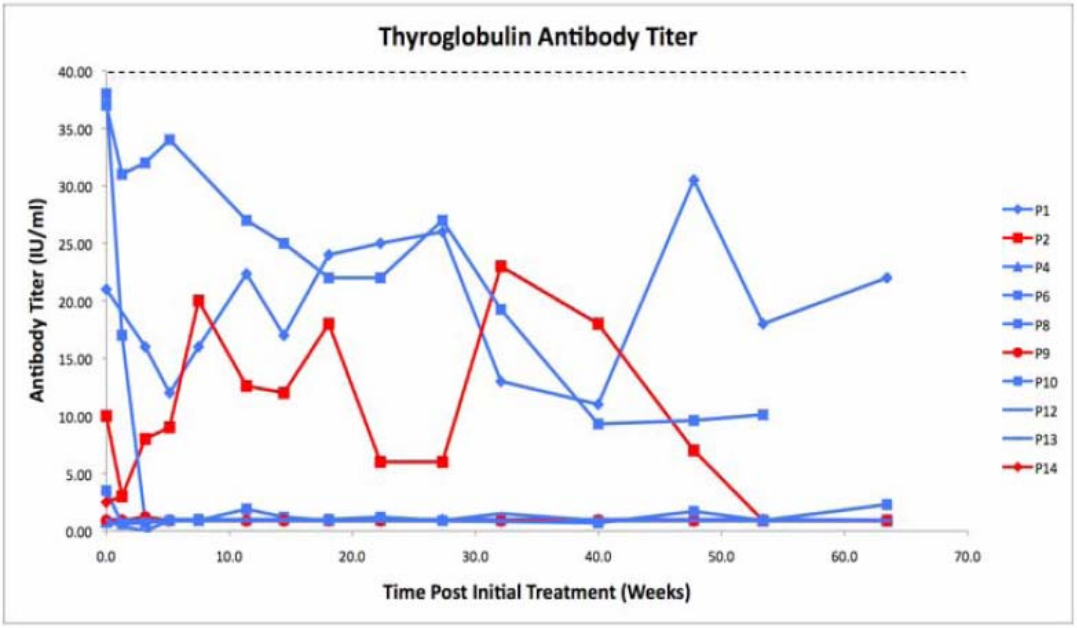




SUPPLEMENTARY DATA

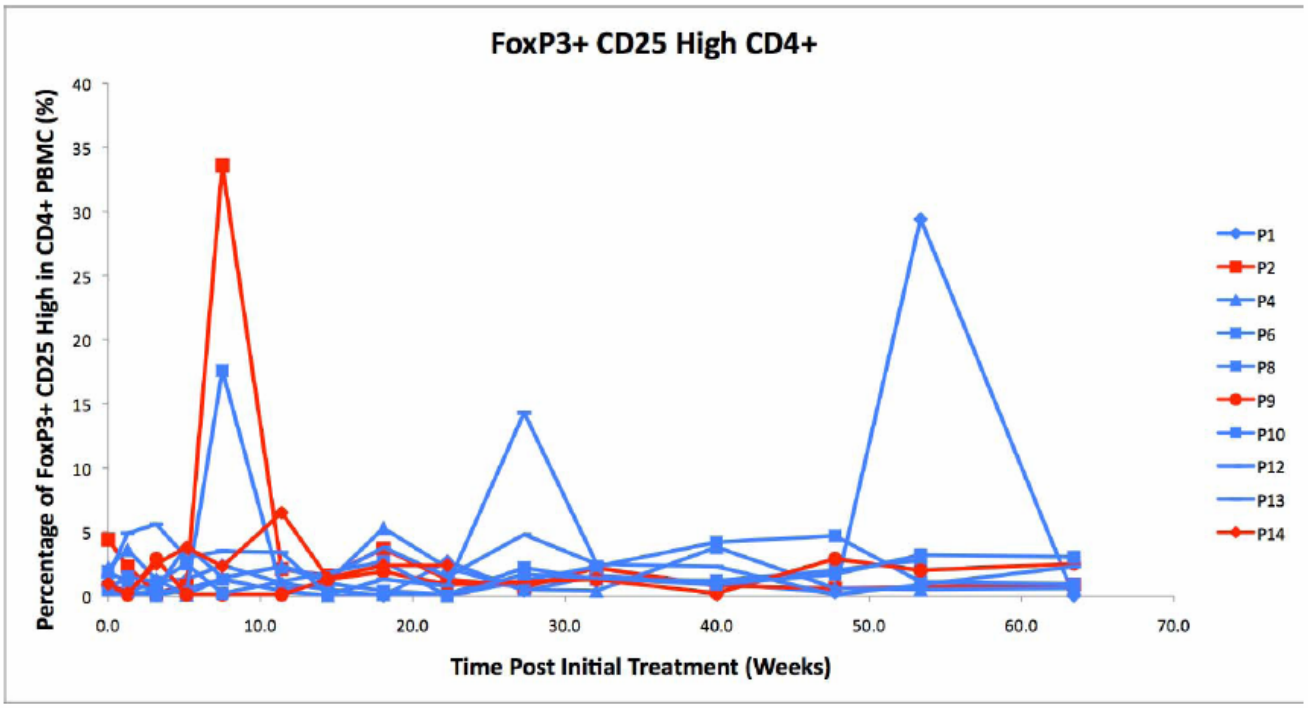
Supplementary Figure 5. Effects of cDC and iDC on serum anti-nuclear and anti-thyroglobulin autoantibody concentration. Autoantibody detection was carried out using commercially-availablity kits as per the manufacturer's instructions by the CTRC hematology laboratory of UPMC using thawed frozen serum samples obtained from each patient at each of the specified visits (refer to graph). The blue-coloured symbols represent the iDC recipients and those in red the cDC recipients. The legend to the right indicates the symbol associated with each individual patient. The dashed lines at the top of the graphs indicate the lowest value considered to be clinically-relevant in standard reference. Week 0 is considered baseline, pre-treatment.





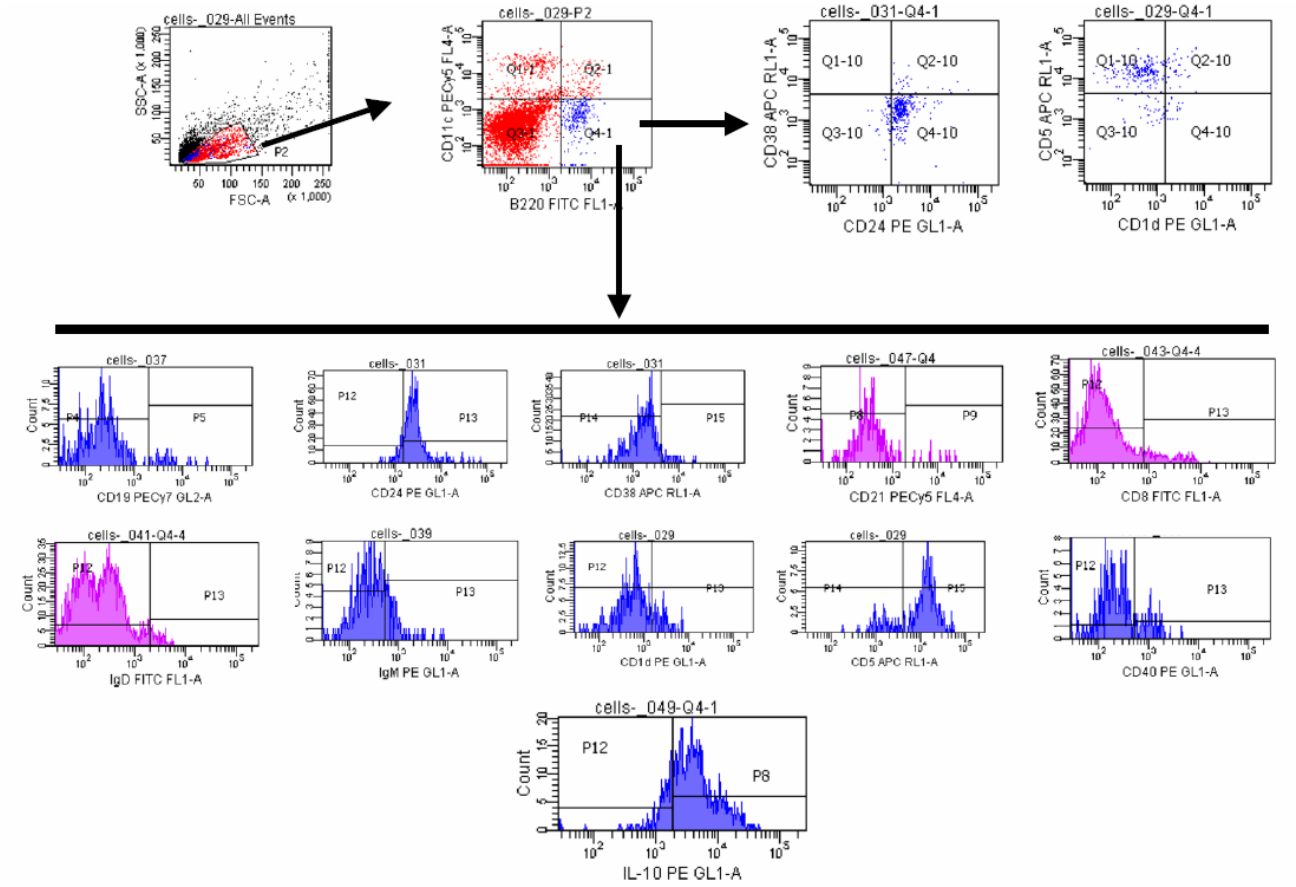
SUPPLEMENTARY DATA

Supplementary Figure 6. Foxp3 Treg frequency in DC recipients. As part of the hematologic monitoring, the frequency of CD4+ $CD25^{HIGH}$ Foxp3+ T-cells in PBMC was measured by FACS at baseline (Week 0) and at each of the weeks of the trial shown in the x-axis. The graph shows the frequency of CD4+ $CD25^{HIGH}$ Foxp3+ T-cells in PBMC at each week during the trial. The red-colored symbols indicate cDC recipients and the black colored symbols represent the iDC recipients. The legend to the right shows the symbols that correspond to each individual patient (P).



SUPPLEMENTARY DATA

Supplementary Figure 7. Characterisation of B-cells upregulated in frequency in response to DC administration by multiparameter flow cytometry. Freshly-isolated PBMC were stained with antibodies specific for human B220 and CD11c along with one of a panel of antibodies to further define the phenotype of the cells. Table 2 lists the relative levels of each marker as well as the percentage of the specific marker-positive cells in the B220+ CD11c- and total PBMC population. All analyses and measurements were carried out with appropriate isotype controls included. The FACS data shown are representative of five genetically-discordant, normal healthy adult individuals selected at random.



SUPPLEMENTARY DATA

Supplementary Figure 7 (cont'd)