Supplemental Material

Safety and Preliminary Efficacy of ORBCEL-M Cell Therapy in Diabetic Kidney Disease: The Multicenter, Randomized, Placebo-controlled NEPHSTROM Trial

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SUPPLEMENTAL RESULTS

Rationale for cohort 1 final analysis

This report provides the final safety and preliminary efficacy analysis for the first cohort of 16 patients with progressive DKD who were randomized in double-blind fashion to receive an infusion of ORBCEL-M at the lowest planned dose (80×10^6 cells) or a placebo infusion. The initial study protocol included two additional cohorts of 16 patients to be completed sequentially. These were to receive an intermediate dose (ORBCEL-M 160×10^6 cells or placebo) and a high dose (ORBCEL-M 240×10^6 cells or placebo) of the trial intervention. Once follow-up of the first dose cohort was completed, the Data Safety Monitoring Board (DSMB) reviewed the relevant data and provided the green light to activate the intermediate dose cohort. Following this, 13 of an intended 16 eligible patients were randomized, treated and are undergoing follow-up at the time of writing. However, due to COVID-19-related delays in enrolment to this second cohort, the approved shelf-life of remaining doses of cryopreserved investigational product for the cohort was exceeded and no further enrolment was possible. For this reason, the NEPHSTROM Sponsor and Trial Steering Committee made the decision to close the trial for further enrolment. As the 13 patients enrolled into the second cohort had completed their treatment and were continuing to be monitoring in double blind fashion, it was concluded that an unblinded analysis of data from the fully completed first cohort would yield important information regarding the safety and preliminary efficacy 80×10^6 cell dose without biasing the subsequent analysis of data for the partially-enrolled second cohort once it is completed.

SUPPLEMENTAL TABLE 1. *List of antibodies and the relative fluorochrome (as Format).*

| 1. Panel for CD4+ and CD8+ T cell, B cell, NK and monocyte subpopulation		
Antibody	*Clone*	*Format*
CD45	HI30	BUV395
CD3	HIT3a	BV510
CD19	HIB19	PECy7
CD27	M-T271	APCR700
IgD	IA6-2	FITC
IgM	G20-127	BV786
CD24	ML5	PE
CD38	HIT2	APC
CD16	B73.1	BB700
CD56	B159	BV650
CD11b	D12	BV711
CD14	M5E2	BUV737
CD64	10.1	PE-CF594
CX3CR1	2A9-1	BV421
HLA-DR	G46-6	BV605
CD33	WM-53	PE-Cy5
Additional reagents: Via-Probe or in alternative 7-AAD, Brillant Stain Buffer and Stain Buffer		
2. Panel for CD4+ Tregs		
Antibody	*Clone*	*Format*
CD45	HI30	BUV395
CD3	HIT3a	BV510
CD4	SK3	BB700
CD8	RPA-T8	BV711
CD45RA	HI100	PECy7
CD45RO	UHCL1	BV786
CD25	(MA251)	BV421
FOXP3	236A/E7	AF647
CD127	HIL-7R-M21	APC-R700
HLA-DR	G46-6	BV605
HELIOS	22F6	PE
CD197(CCR7)	150503	PE-CF594
CD62L	DREG-56	BV650
CD28	CD28.2	BUV737
CD95	DX2	PECy5
Additional reagents: Fixable Viability Stain 520, Transcription Factor Buffer Set, Brillant Stain Buffer, Stain Buffer		
3. Panel for Dendritic cells		
Antibodies	*Clone*	*Format*
CD45	HI30	BUV395
Lineage Cocktail 1(lin 1)	multiple	FITC
HLA-DR	G46-6	BV605
CD1c	F10/21A3	Alexa647
CD141	1A4	BB700
CD303	V24-785	BV421
CD123	9F5	PE
CD11c	B-ly6	BV711
CD33	WM-53	PE-Cy5
Additional reagents: Via-Probe or in alternative 7-AAD, Brillant Stain Buffer and Stain Buffer		

SUPPLEMENTAL FIGURE 1. *Correlations between percentages of Tregs or CD45RA⁻RO⁺ memory Tregs and serum concentrations of inflammatory mediators in the overall study cohort.* Correlations between percentages of Tregs (A) or CD45RA⁻RO⁺ memory Tregs (B) within peripheral blood CD3⁺CD4⁺ T cells and serum TNFR1 concentrations in the overall study cohort (r = -0.3690 and -0.4024, respectively; both *P*<0.001). Correlations between percentages of Tregs (C) or CD45RA⁻RO⁺ Tregs (D) within peripheral blood CD3⁺CD4⁺ T cells and serum NGAL concentrations in the overall study cohort (r = -0.3879 and -0.3907, respectively; both *P*<0.001). TNFR1, tumor necrosis factor receptor 1; NGAL, neutrophil gelatinase-associated lipocalin.
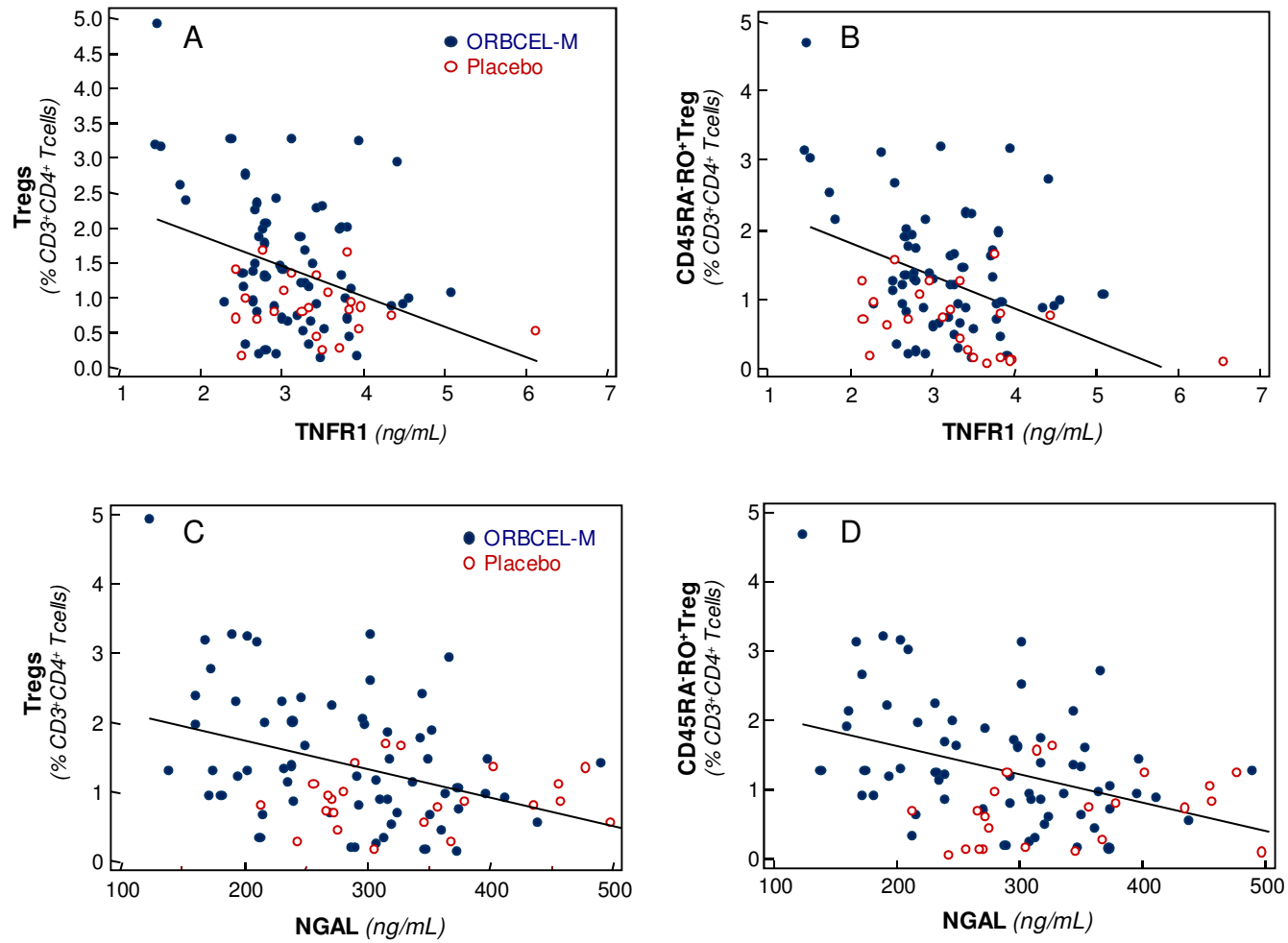
SUPPLEMENTAL FIGURE 2. *Correlations between percentages of Tregs or CD45RA-RO+ memory Tregs and estimated glomerular filtration rate in the overall study cohort.* Correlations between percentages of Tregs within peripheral blood CD3+CD4+ T cells and glomerular filtration rate estimated with CKD-EPI (A) or MDRD (B) equations in the overall study cohort (r = 0.2235, P=0.035 and r = 0.2949, P<0.005, respectively). Correlations between percentages of CD45RA-RO+ memory Tregs within peripheral blood CD3+CD4+ T cells and glomerular filtration rate estimated with CKD-EPI (C) or MDRD (D) equations in the overall study cohort (r = 0.2578, P=0.015 and r = 0.3313, P<0.002, respectively). CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration equation; MDRD, Modification of Diet in Renal Disease equation.
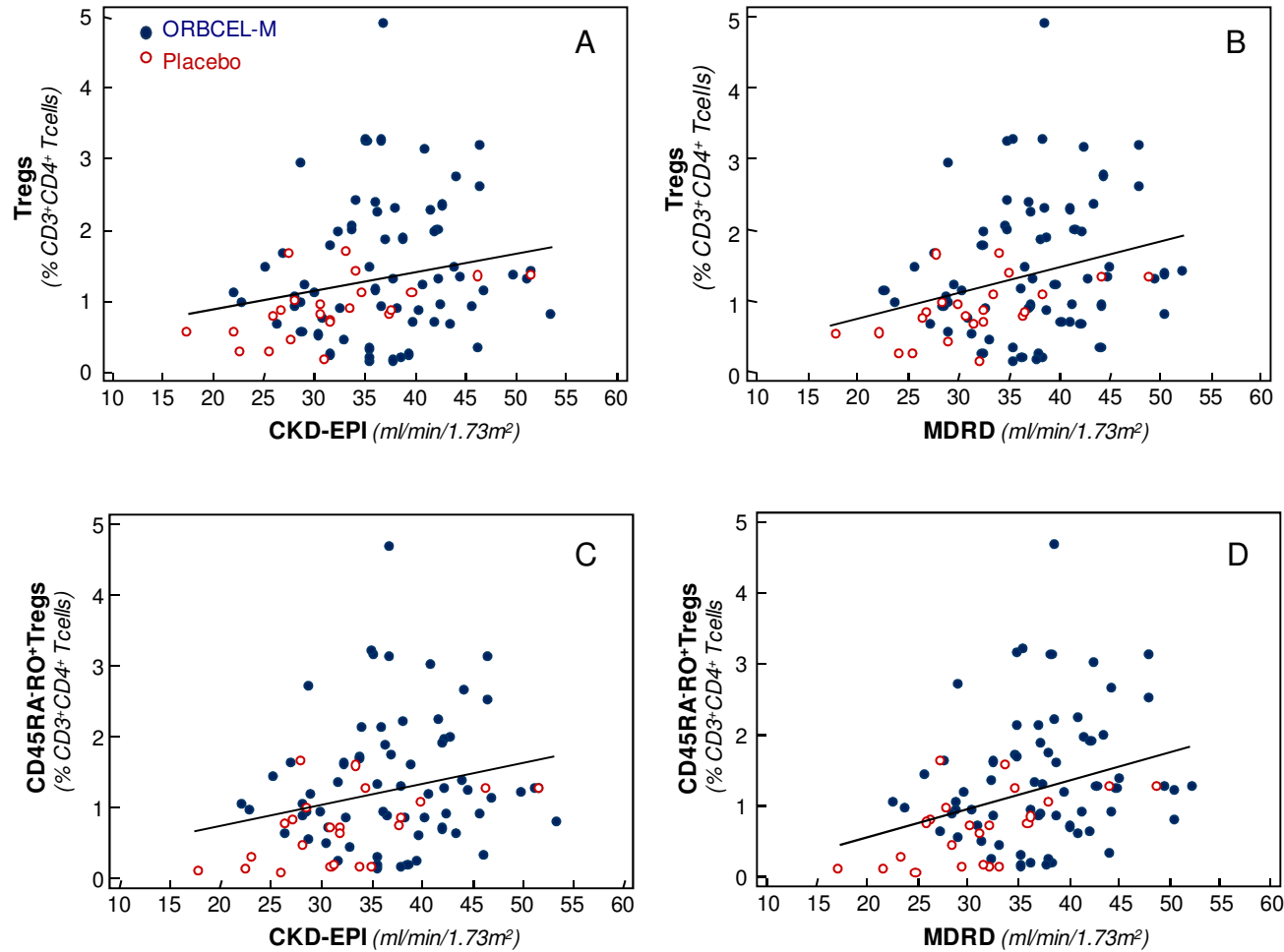
SUPPLEMENTAL FIGURE 3. *Correlations between serum concentrations of inflammatory mediators and measured or estimated glomerular filtration rate in the overall study cohort.* Correlations between serum TNFR1 concentrations and glomerular filtration rate measured by iohexol plasma clearance (A) or estimated by CKD-EPI (B) or MDRD (C) equations in the overall study cohort (r = -0.3929, *P*=0.002, r = -0.5493, *P*<0.001 and r = -0.5928, *P*<0.001, respectively). Correlations between serum NGAL concentrations and glomerular filtration rate measured by iohexol plasma clearance (D) or estimated by CKD-EPI (E) or MDRD (F) equations in the overall study cohort (r = -0.5932, *P*<0.001, r = -0.3486, *P*=0.001 and r = -0.3626, *P*=0.001, respectively). CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration equation; MDRD, Modification of Diet in Renal Disease equation; NGAL, neutrophil gelatinase-associated lipocalin; TNFR1, tumor necrosis factor receptor 1.
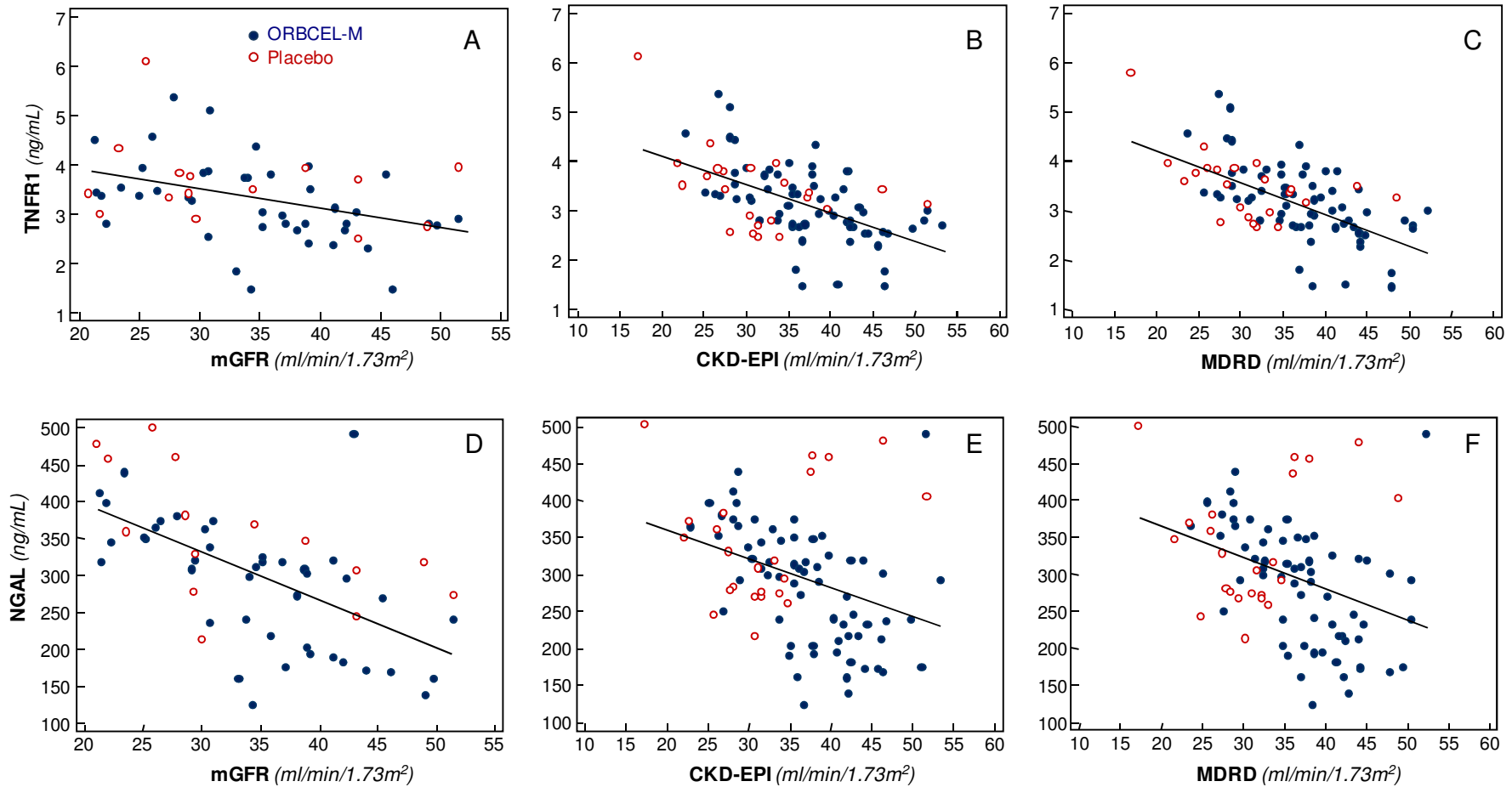
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