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Supplementary Information for

Immune Tolerance in Multiple Sclerosis and Neuromyelitis Optica by Peptide-Loaded Tolerogenic Dendritic Cells in a Phase 1b Trial.

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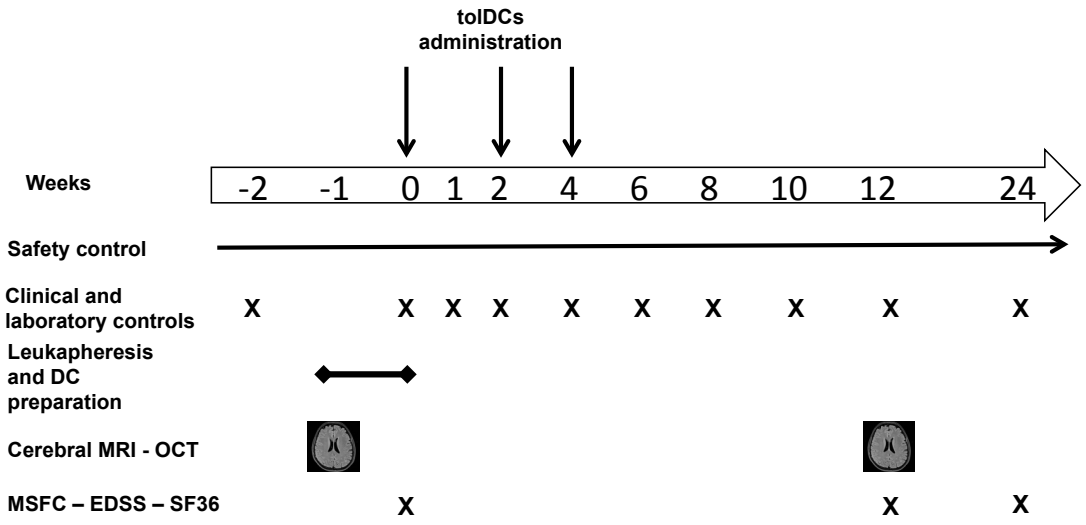

Figure S1: Clinical trial design of tolDC therapy in patients with MS and NMO. Patients were screening for inclusion and exclusion criteria as well for MRI disease activity and blood test 2 weeks before onset of therapy and white cells were collected by leukapheresis for tolDC preparation. On day 0 (baseline visit) patients performed the clinical visit, including disability scales (EDSS and MSFC) and received the first dose of tolDC (representing 1/3 of the total dose). Patients received the second dose 2 weeks later and the third dose on week 4 of the trial. Patients were followed until week 12 for efficacy and immunological assessments and until week 24 for safety assessment (extension safety assessments were performed by month 12, 18 and 24 if available). Brain MRI and OCT were conducted during the screening and by week 12. Blood samples were collected weekly for the first two weeks, every 2 weeks until week 12.

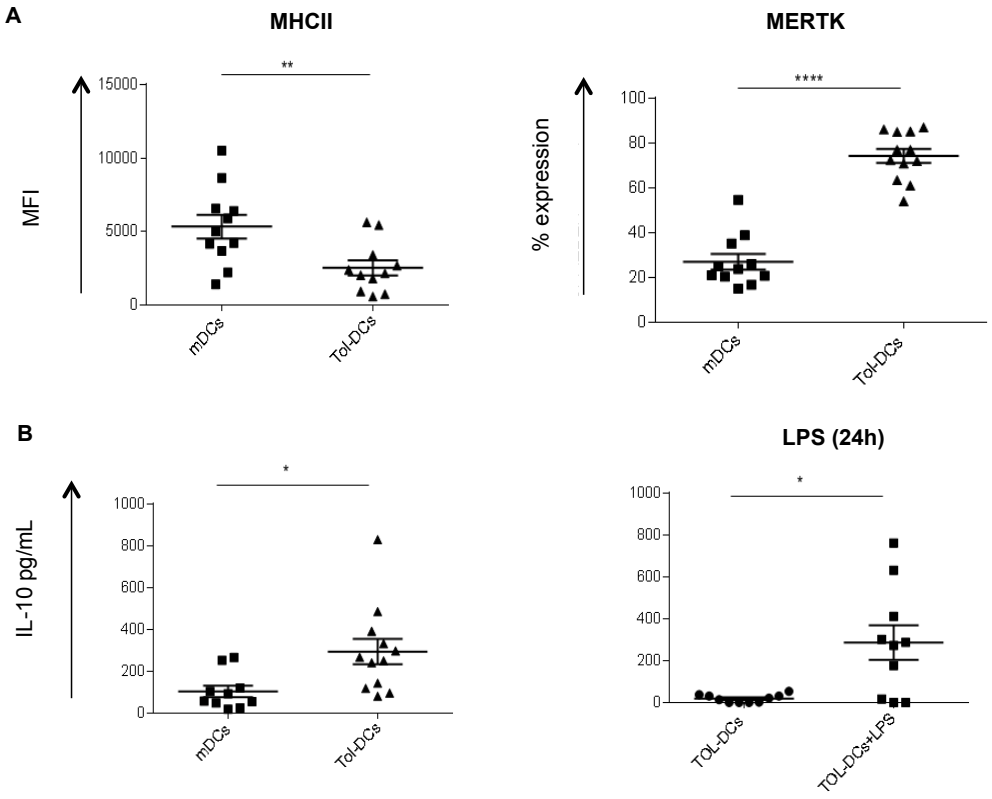

Figure S2: TolDCs characterization and stability. Clinical grade produced tolDCs from each patient were characterized assessing for their phenotype, cytokine production, and immunogenicity. A) semi-mature phenotype of tolDCs as shown by the lower expression of MHCII and increased expression of the glucocorticoid-induced receptor MERTK compared to mature DCs. B) clinical grade generated tolDCs produced higher amounts of the IL-10 at baseline and after LPS challenge compared to mature DCs.

Table S1. Peptides and sequence

Peptide	Sequence	Aminoacids sequence
MBP1	MBP 13-32	KYLATASTMDHARHGFLPRH
MBP2	MBP 83-99	ENPVVHFFKNIVTPRTP
MBP3	MBP 11-129	LSRFSWGAEGQRPGFGYGG
MBP4	MBP 146-170	AQGTLSKIFLGGRDSRSGSPMARR
MOG1	MOG 1-20	GQFRVIGPRHPIRALVGDEV
MOG2	MOG 35-55	MEVGWYRPPFSRVVHLYRNGK
PLP1	PLP 139-154	HCLGKWLGHPDKFVGI
AQP4	AQP4 63-76	EKPLPVDMVLISLC

Table S2. Panel for flow cytometry and antibodies

1. **lymphocyte subpopulations**: CD3 – CD4 – CD8 – CD19
   - CD3 (FITC) – BD 345763
   - CD4 (PE Cy7) – BD 560649
   - CD8 (APC) - BD 555369
   - CD19 (PerCP-C5.5) – BD 332780
2. **Monocyte and NK cells subpopulations**: CD3 – CD56 – CD14
   - CD3 (FITC) – BD 345763
   - CD14 (PE Cy7) – BD 557742
   - CD56 (APC-Cy7) - BioLegend 318332
3. **T-cytotoxic and NK cell populations**: CD4 - CD8 – CD56 – CD161
   - CD4 (PE Cy7) – BD 560649
   - CD8 (APC) - BD 555369
   - CD56 (APC-Cy7) - BioLegend 318332
   - CD161 (PerCP-Cy5.5) – BioLegend 339908
4. **Treg subpopulations**: CD4 – CD25high – FoxP3 – IL10
   - CD4 (PE Cy7) – BD 560649
   - IL-10 (PE) – BD 559330
   - FoxP3 (Alexa Fluor 647/APC) – BD 560045
   - CD25 (PerCP) - Biolegend 356112
5. **T-helper subpopulations**: CD4 – IFNgamma – IL4 – IL17
   - CD4 (PE Cy7) – BD 560649
   - IFN gamma (PE) – BD 340452
   - IL-17 (PerCP 5.5) – BD 560799
   - IL-4 (APC) – BD 554486
6. **Encephalitogenic T cells expressing GM-CSF**: CD4 – CD8 – GM-CSF
   - CD4 (PE Cy7) – BD 560649
   - CD8 (APC) - BD 555369
   - GM CSF (PE) – BD 554507
7. **T cells: naive, central memory, effector and effector memory cells**
   - Viability (FITC) - - L34969
   - CD3 (V450) – BD 560365
   - CD4 (PerCP Cy5.5) – BD 552838
   - CD8 (V500) – BD 560774
   - CCR7 (PE) – MILTENYI 130-099-362
   - CD45RA (PE Cy7) – BD 561216
   - CD45RO (APC) – BD 559865
   - ICOS (APC Cy7) - BD 313529

Naïve	CD3+CD4/8+ CCR7+ CD45RA+CD45RO-
Central memory	CD3+CD4/8+ CCR7+ CD45RA-CD45RO+
Effector	CD3+CD4/8+ CCR7- CD45RA+CD45RO-
Effector memory	CD3+CD4/8+ CCR7- CD45RA-CD45RO+

Table S3. Retina thickness by Optical Coherent Tomography at baseline and week 12. Results are expressed in μm. NA refers to lack of valid measurements fulfilling OSCAR-IB criteria because presence of nistagmus, severe vision loss or concomitant ophthalmologic diseases.

	Baseline				Week 12			
ID	**RNFL OD**	**RNFL OS**	**MV OD**	**MV OS**	**RNFL OD**	**RNFL OS**	**MV OD**	**MV OS**
MS01	78	73	263	259	78	73	267	261
MS02	96	91	276	272	96	92	278	271
MS03	NA	NA	NA	NA	NA	NA	NA	NA
MS04	105	103	267	267	106	104	267	265
MS05	69	81	269	274	69	80	273	275
MS06	98	96	292	292	99	97	290	286
MS07	115	111	262	275	114	110	263	273
MS08	62	52	289	287	65	53	290	288
NMO01	78	91	278	280	78	89	276	283
NMO02	42	NA	244	NA	NA	NA	NA	NA
NMO03	NA	NA	NA	NA	NA	NA	NA	NA
NMO04	43	47	232	228	NA	NA	NA	NA

References

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