

CD4⁺ T-cell alloreactivity after haploidentical hematopoietic stem cell transplantation

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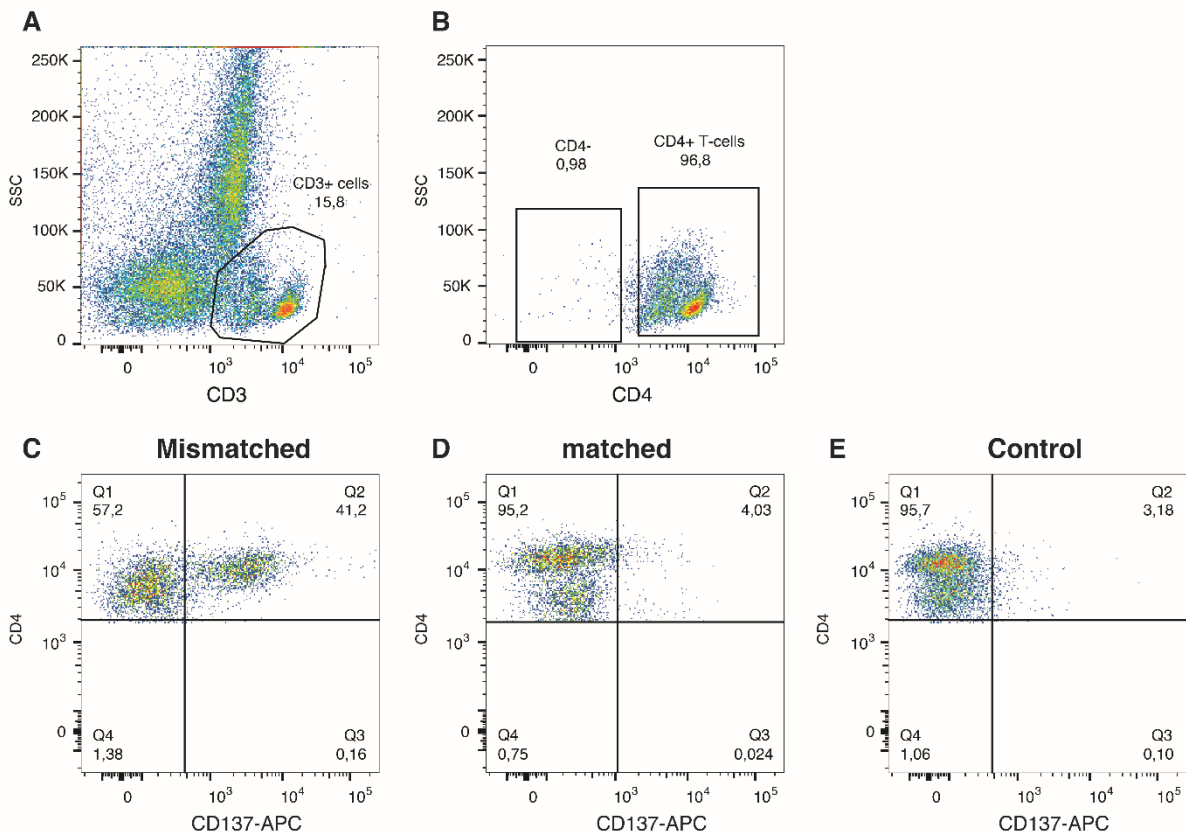
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SUPPLEMENTARY APPENDIX

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Supplemental Figure 1



Supplemental Figure 1: Mismatched HLA-class II-specific amplification - alloreactivity assay set up and gating strategy.

Patient ID1, PBMC from 1 month post dUCBT were processed using HeLa cells transduced with mismatched HLA-class II alleles DRB1*07:01 and subsequently assayed for HLA-class II allele specific upregulation of CD137 by FCM after stimulation with HeLa cell lines transduced with the (mis)matched or control empty HeLa cell. Analysis was performed by using a sequential gating strategy, i.e., Step 1: gating on T-cells on basis of their CD3+, SSClow characteristics [plot A], excluding stimulator HeLa cells; Step 2: selection of CD4+ T-cells by plotting CD4+ within CD3+ against SSClow. Step 3: the events in Gate 2 are plotted in a CD4 vs CD137 plot and applying quadrant statistics. Inserted numbers represent the proportions of CD4+ T-cells expressing the activation markers [Q2]. CD4+ T-cell activation towards mismatched DRB1*07:01 [Plot C], matched DQB1*03:01 [Plot D] and control 'empty' [Plot E] HeLa cells are illustrated.

Abbreviations: PBMC, peripheral blood mononuclear cells; HLA, human leukocyte antigen; FCM, flow cytometry