Treatment with bortezomib of human CD4+ T cells preserves natural regulatory T cells and allows the emergence of a distinct suppressor T-cell population

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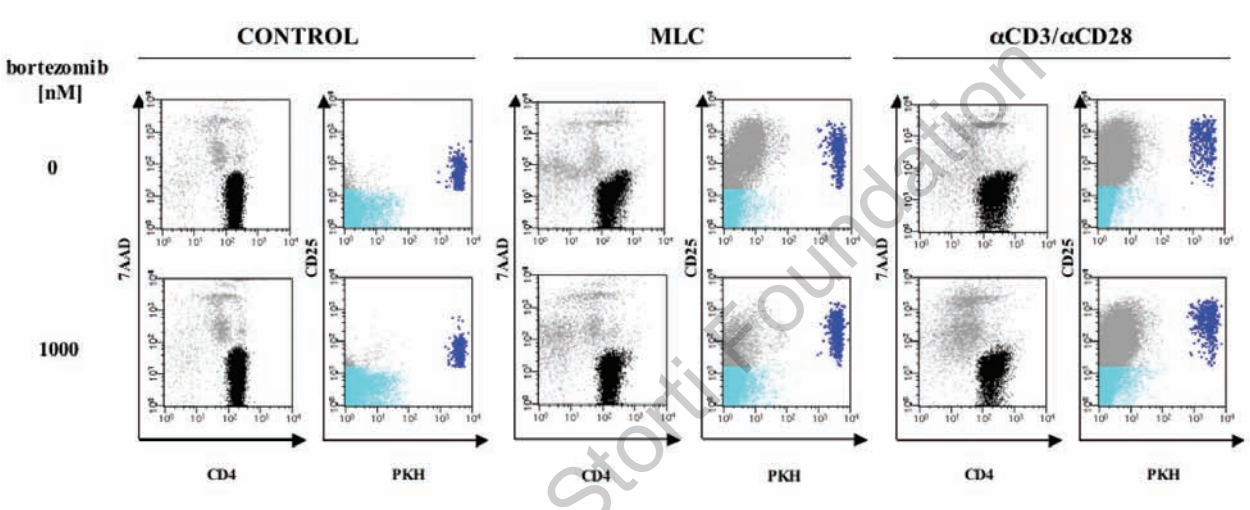
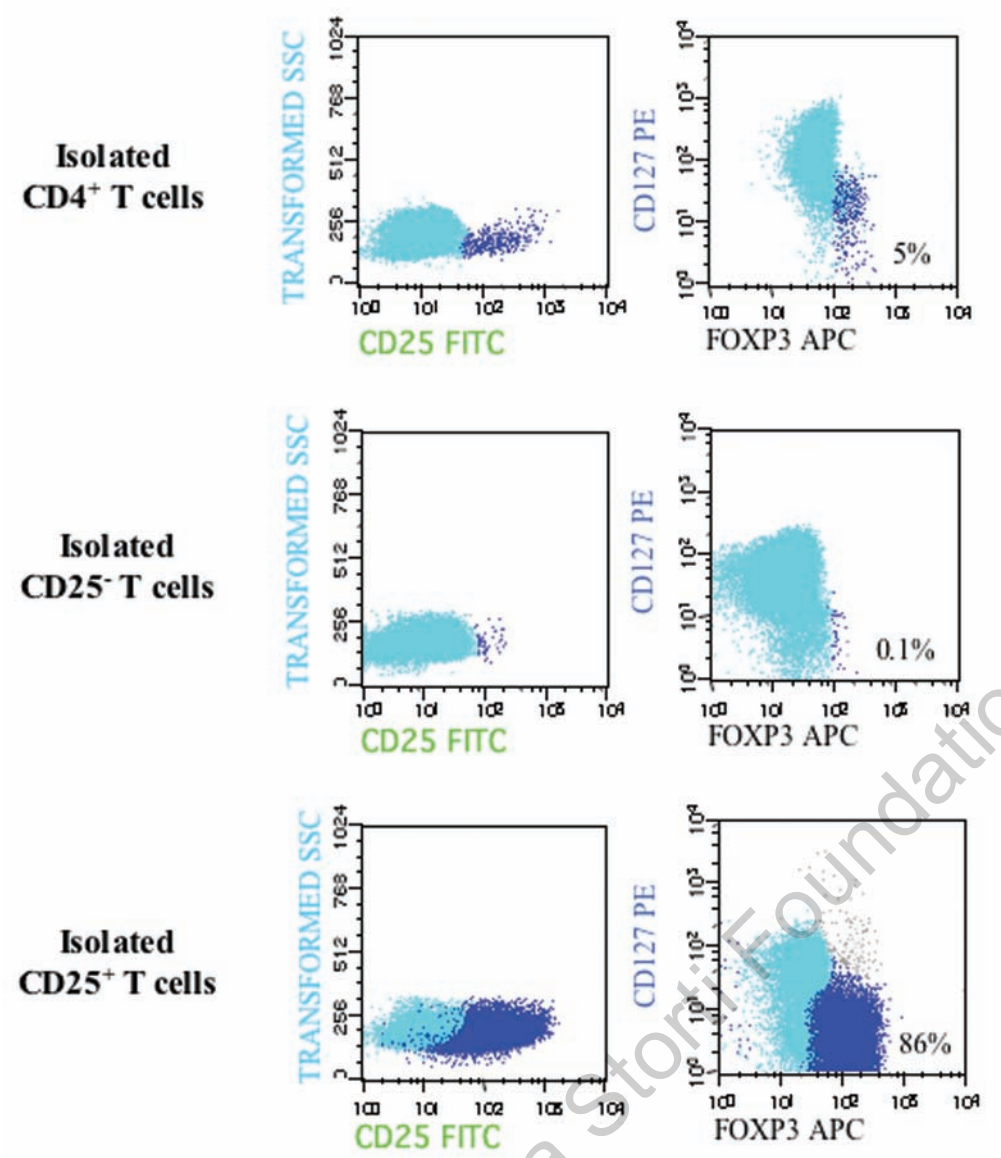
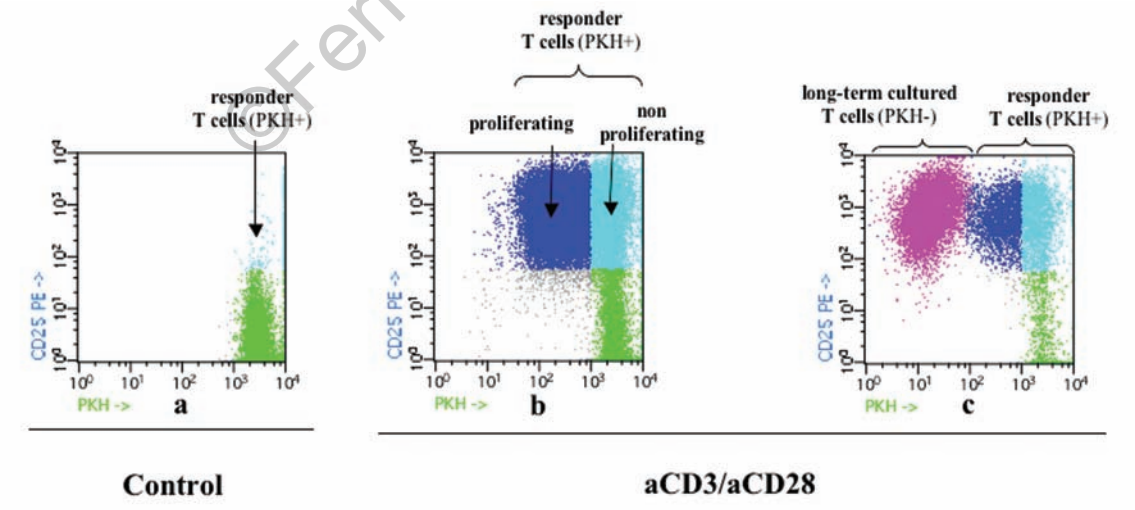
Citation: Blanco B, Pérez-Simón JA, Sánchez-Abarca LI, Caballero-Velazquez T, Gutierrez-Cossío S, Hernández-Campo P, Díez-Campelo M, Herrero-Sanchez C, Rodriguez-Serrano C, Santamaría C, Sánchez-Guijo FM, del Cañizo C, and San Miguel JF. Treatment with bortezomib of human CD4+ T cells preserves natural regulatory T cells and allows the emergence of a distinct suppressor T-cell population. Haematologica 2009; doi:10.3324/haematol.2008.005017



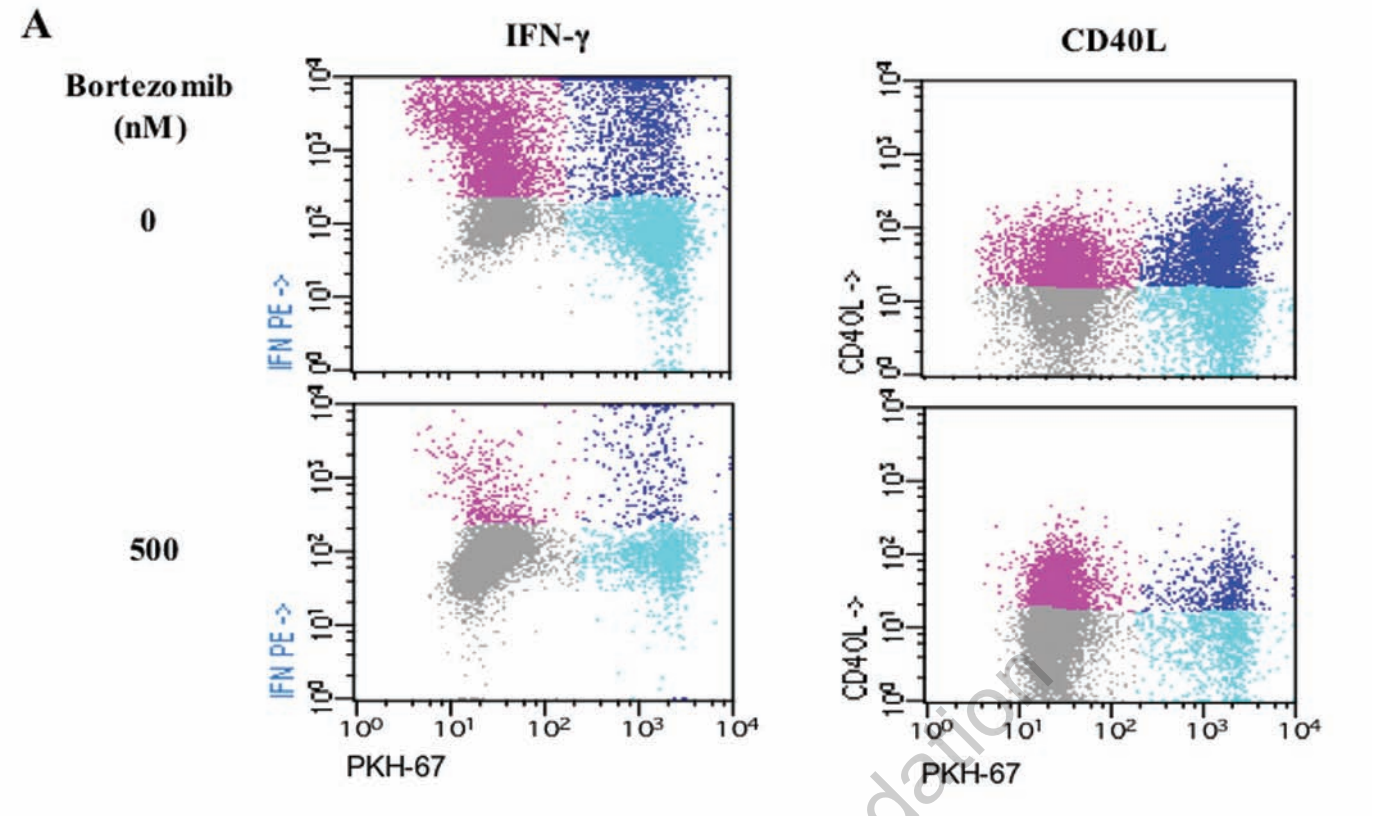
Online Supplementary Figure S1. Viability of the different types of CD4+ T cells: regulatory (CD25+ PKH+), resting conventional (CD25- PKH-) and activated conventional (CD25+ PKH-) T cells, assessed by the percentage of 7AAD- cells of each subpopulation



Online Supplementary Figure S2. Analysis of CD25, FOXP3 and CD127 expression by total $CD4^+$, $CD4^+CD25^-$ or $CD4^+CD25^+$ T cells separated by immunomagnetic selection based on the expression of CD4 and CD25.



Online Supplementary Figure S3. Suppression assays: proliferation and CD25 expression. (A) Schematic representation of 4-day suppression assays analysis by flow cytometry: (a) unstimulated PKH-stained responder T cells; (B) aCD3/aCD28 stimulated PKH-stained responder T cells; (C) aCD3/aCD28 stimulated PKH-stained responder T cells co-incubated with non PKH-stained long-term cultured cells, among which we can distinguish: long-term cultured cells (violet), proliferating $CD25^+$ responder T cells (blue), non-proliferating $CD25^+$ responder T cells (cyan) and non-proliferating $CD25^-$ responder T cells (green).



Online Supplementary Figure S4. Suppression assays: IFN-g and CD40L production after co-culture of responder plus long-term cultured T cells. (A) IFN-γ and CD40L intracytoplasmic expression by both responder (PKH^+) and long-term cultured (PKH^-) T cells. One experiment of five is shown.