Regulatory dendritic cells correlate with an altered T cell distribution and regulatory T cell phenotype in metastatic lymph nodes of NSCLC patients.

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INTRODUCTION

Dendritic cells (DCs) are considered to be the most important professional population of antigen-presenting cells and play a crucial role in activation of the immune system. However, the proper function of DCs is altered by the tumor microenvironment (TME). Despite the progress in anticancer immunotherapy, there are still many unresolved issues. Among them the immunomodulatory properties of DCs in lung cancer are of interest.

AIM OF THE STUDY

The aim of the study was to analyze the PD-1+ PD-L1+ CD80- DCs subset, described as regulatory dendritic cells (DCregs) and to investigate correlations between the presence of DCregs and changes in T cell frequencies and immunomodulatory molecules in metastatic and non-metastatic lymph nodes (LNs) aspirates of NSCLC patients.

[1] Schmidt SV, Nino-Castro AC, Schultze JL (2012) Regulatory dendritic cells: there is more than just immune activation. Front Immunol 3:274. doi:10.3389/fimmu.2012.00274
[2] Krempski J, Karyampudi L, Behrens MD, Erskine CL, Hartmann L, Dong H, Goode EL, Kalli KR, Knutson KL (2011) Tumor-infiltrating programmed death receptor-1+ dendritic cells mediate immune suppression in ovarian cancer. J Immunol
[3] Ma Y, Shurin GV, Gutkin DW, Shurin MR (2012) Tumor associated regulatory dendritic cells. Semin Cancer Biol 22 (4):298-306.

RESULTS
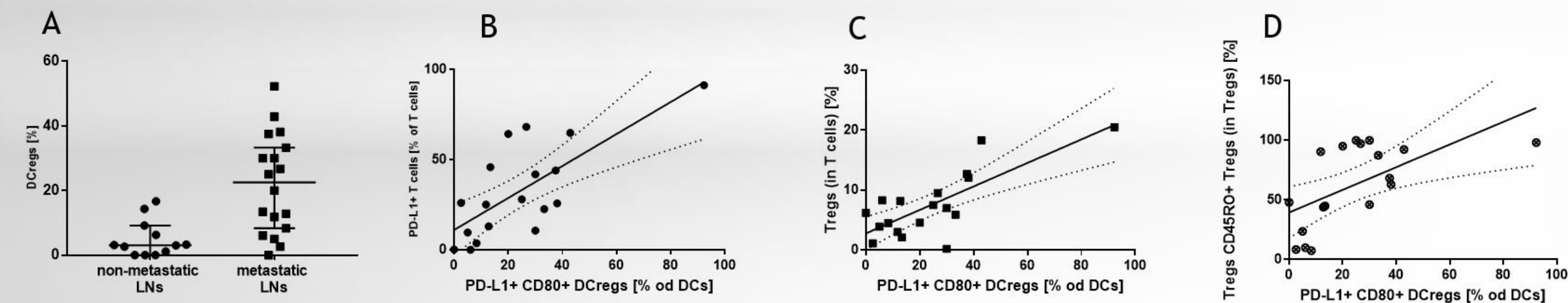
A) We noticed a higher percentage of DCregs in the metastatic than in the non-metastatic LNs (median = 22.5% vs. 3.1%, $p<0.05$ Mann-Whitney test).
B) In metastatic LNs percentage of DCregs positively correlated with the percentage of PD-1+ T cells. (r=0.5307).
C) In metastatic LNs percentage of DCregs positively correlated with the percentage of Tregs. (r=0.5897)
D) In metastatic LNs percentage of DCregs positively correlated with the percentage of CD45RO+ Tregs (activated subset of highly proliferative Tregs)*

*Booth NJ et al. Different Proliferative Potential and Migratory Characteristics of Human CD4+ Regulatory T Cells That Express either CD45RA or CD45RO. J Immunol April 15, 2010, 184 (8) 4317-4326

CONCLUSION

Our results confirm the utility of flow cytometric analysis of EBUS/TBNA samples to assess the interaction between immune cells in TME. DCregs strongly associates with an altered T cell distribution and immunosuppressive phenotype in metastatic LNs of NSCLC patients. It can be assumed that DCregs are involved in the suppression of anti-tumor response, which could be of importance for the response upon immunotherapy.

METHODS

LNs aspirates were obtained during diagnostic EBUS TBNA procedure of 30 NSCLC patients. DCregs and T cell characteristics were determined by multiparameter flow cytometry.
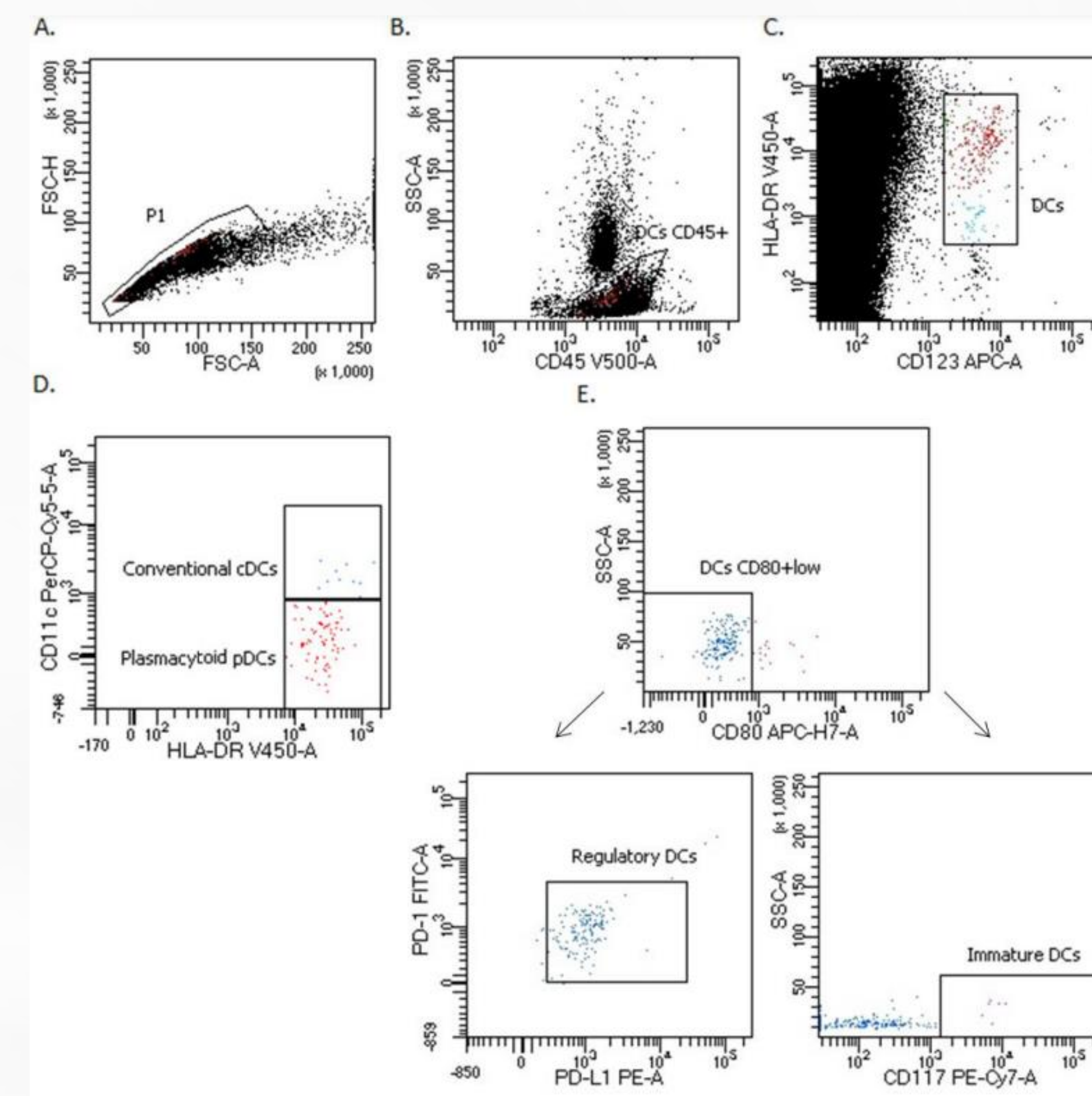
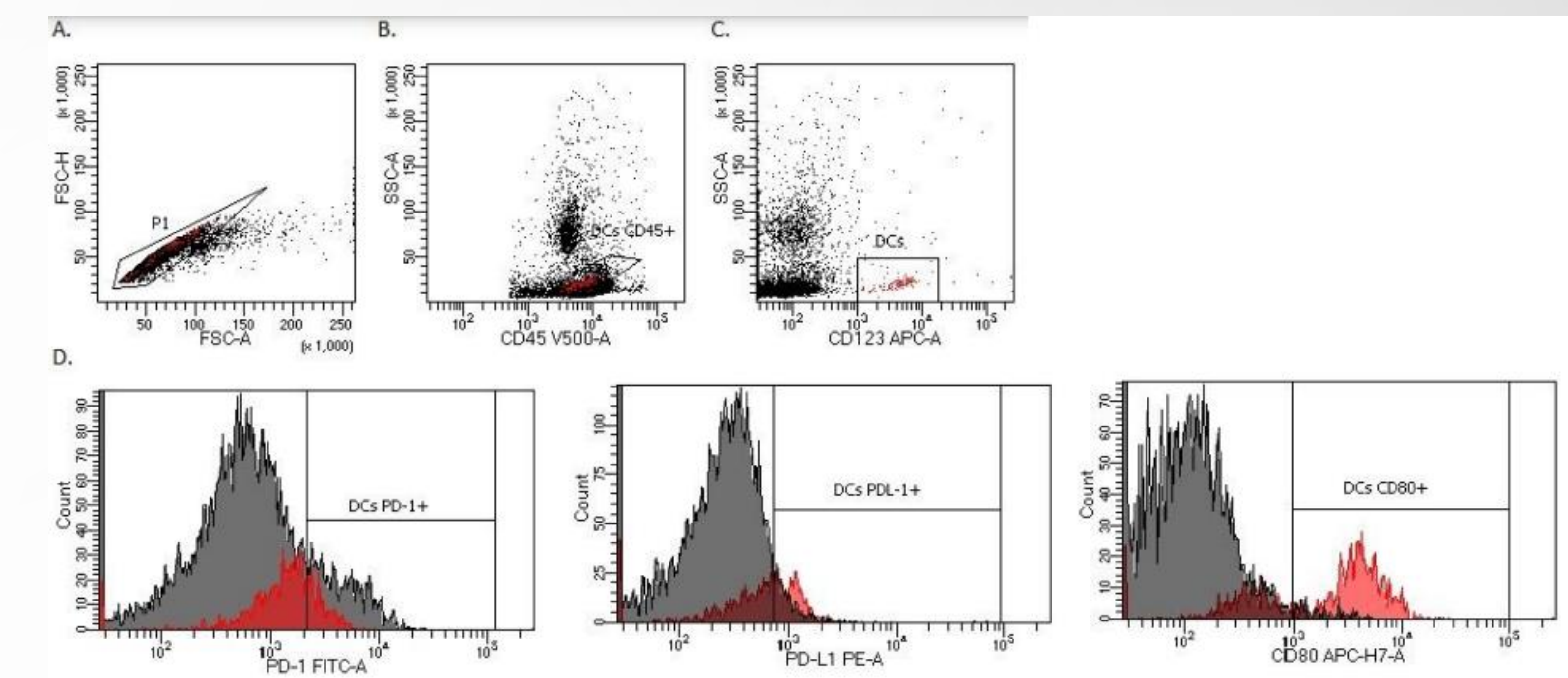Representative dendritic cells (DCs) subsets gating strategy in non-metastatic lymph node aspirate.
(A) FSC-H vs. FSC-A plot: Gating the cells that have an equal area and height, thus removing clumps (greater FSCA relative to FSC-H and debris (very low FSC).
(B) SSC-A vs. CD45 plot: Broad selection of DCs CD45+ based on their SSC-A/CD45+ properties.
(C) HLA-DR vs. CD123 plot: Broad selection of DCs based on their HLADR+/CD123+ properties.
(D) HLA-DR vs. CD11c plot: Broad selection of conventional DCs (red HLADR+/CD11c+) and plasmacytoid DCs (turquoise HLA-DR+/CD11c-) based on their HLA-DR/CD123properties.
(E) SSC-A vs. CD80 plot: Broad selection of DCs CD80+ based on their SSC-A/CD80+ properties (blue) and with this plot selection of regulatory DCs (blue PD-1+/PD-L1+/CD80+low) and immature DCs (purple CD80+low/CD117+).

Representative dendritic cells (DCs) gating strategy with expression of PD-1, PDL-1 and CD80 in metastatic lymph node aspirate.
(A) FSC-H vs. FSC-A plot: Gating the cells that have an equal area and height, thus removing clumps (greater FSC-A relative to FSC-H and debris (very low FSC).
(B) SSC-A vs. CD45 plot: Broad selection of DCs CD45+ based on their SSC/CD45+ properties (red cells).
(C) SSC-A vs. CD123 plot: Broad selection of DCs based on their SSC/CD123+ properties (red cells).
(D) count vs. PD-1/PD-L1 or CD80 histograms: selection of DCs with PD-1, PD-L1 or CD80 expression (red cells- DCs, grey cells- the remaining cells).