Blockade of programmed cell death protein 1 (PD-1) in Sézary syndrome reduces Th2 phenotype of non-tumoral T lymphocytes but may enhance tumor proliferation

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Introduction

Sézary syndrome (SS) is an aggressive leukemic variant of cutaneous T-cell lymphoma (L-CTCL) that arises from malignant clonally derived skin-homing CD4+ T cells. Based on advancements in our under-standing of the mechanisms underlying L-CTCL, boosting the suppressed immune response emerges as a promising strategy in SS management. Immune checkpoint inhibitory molecules have already demon-strated efficacy in a wide spectrum of malignancies. Currently, agents targeting the programmed death-1 (PD-1) axis are under evaluation in L-CTCL. Here we investigated the expression of PD-1 and its ligands, PD-L1 and PD-L2 in blood and skin from patients with L-CTCL.

Materials and methods

Patients and human samples (skin and blood) from the Department of Dermatology of the University Hospital of Zurich, Switzerland, with previously diagnosed L-CTCL (SS) and with unequivocally identifiable Vβ clonal T-cell population in their blood and/or skin were included. Blood samples from healthy individuals served as control. Blood from healthy individuals was obtained from the blood bank of the University Hospital Zurich. Healthy skin (surgical remnants and control margins) was obtained as discarded tissue through the University of Zürich Biobank. Please find the detailed description of the applied methods in the original publication[1] for: (1) Isolation of T cells from human skin, (2) Flow cytometry, (3) Intracellular flow cytometry for IFN-γ and IL-4 , (4) T cell proliferation assay , (5) Immunohistochemistry (IHC), (6) Statistical analysis

Results

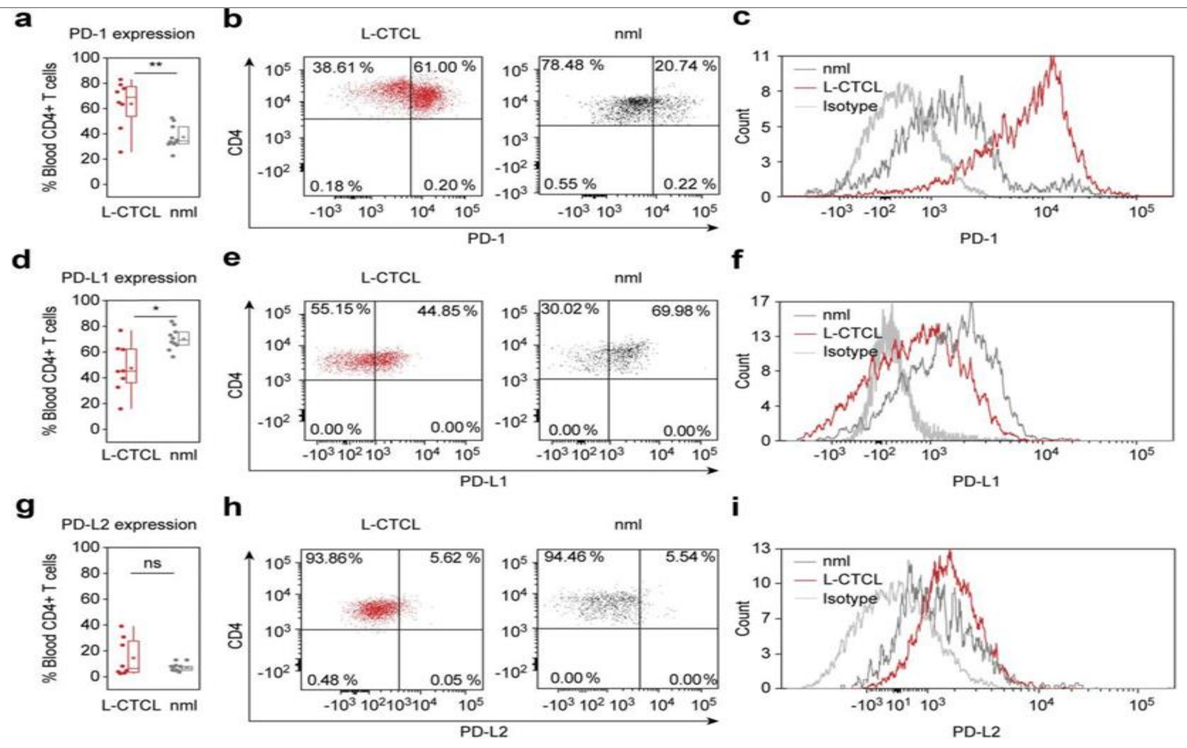

Figure 1. PD-1 is up – while PD-L1 is downregulated in peripheral blood T cells of L-CTCL patients. Percentage of PD-1, PDL-1 and PDL-2 positive cells upon staining with fluorochrome-conjugated monoclonal antibodies was assessed in double CD3- and CD4-positive cells. (a) T helper subset in L-CTCL individuals (n = 8) was characterized with significantly upregulated PD-1 expression compared to the healthy volunteers (n = 10). Representative dot blot (b) and histogram (c) demonstrate increased PD-1 expression on CD4⁺ T cells in blood from patients with L-CTCL, as compared to healthy donors. In contrast to PD-1, PD-L1 (d) showed decreased expression on CD4⁺ T cells in blood from patients with L-CTCL in comparison to healthy donors. Representative dot blot (e) and histogram (f) further visualize the lower PD-L1 expression on CD4⁺ T cells in L-CTCL. The percentage of peripheral blood CD4⁺ T cells positive for PD-L2 was low and did not differ significantly between L-CTCL patients and healthy donors (g). Mean values of percentage PD-L2 positive T lymphocytes (h) and median fluorescent intensity for the same marker (i) were in similar range for the patient and control cohort. Abbreviations: ns: P > .05; *: P ≤ 0.05; **: P ≤ 0.01; nlm: healthy donors.

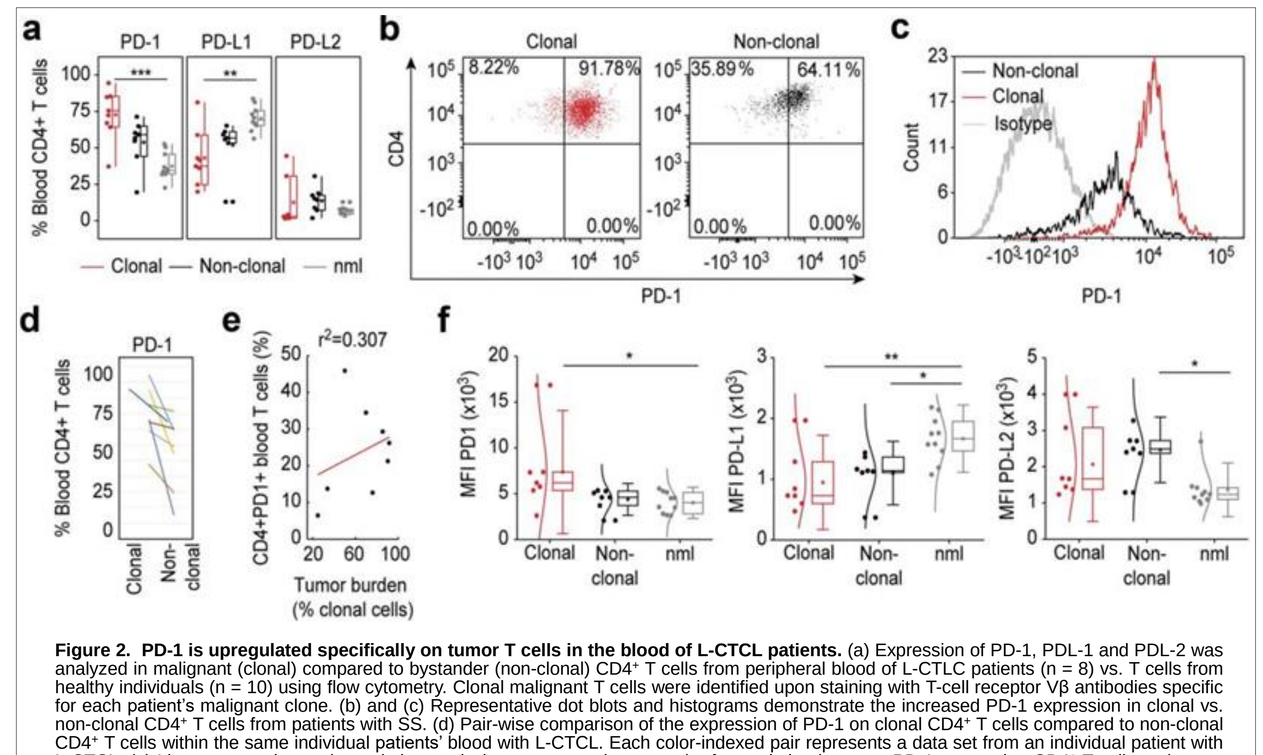

Figure 2. PD-1 is upregulated specifically on tumor T cells in the blood of L-CTCL patients. (a) Expression of PD-1, PDL-1 and PDL-2 was analyzed in malignant (clonal) compared to bystander (non-clonal) CD4⁺ T cells from peripheral blood of L-CTLC patients (n = 8) vs. T cells from healthy individuals (n = 10) using flow cytometry. Clonal malignant T cells were identified upon staining with T-cell receptor Vβ antibodies specific for each patient's malignant clone. (b) and (c) Representative dot blots and histograms demonstrate the increased PD-1 expression in clonal vs. non-clonal CD4⁺ T cells from patients with SS. (d) Pair-wise comparison of the expression of PD-1 on clonal CD4⁺ T cells compared to non-clonal CD4⁺ T cells within the same individual patients' blood with L-CTCL. Each color-indexed pair represents a data set from an individual patient with L-CTCL. (e) Linear regression and correlation analysis to measure the strength of association between PD-1 expressing CD4⁺ T cells and tumor burden, defined by % clonal T cells of all CD4⁺ T cells. (f) Mean fluorescent intensities (MFIs) of the stainings for PD-1 and its ligands in malignant vs. bystander and normal peripheral blood CD4⁺ T cells were assessed. Clonal malignant T cells were definitively identified by staining with T-cell receptor Vβ antibodies specific for each patient's malignant clone and showed significant increase of PD-1 expression compared to the control CD4⁺ T lymphocytes. On the contrary, PD-L1 was downregulated in L-CTCL patients on both clonal and non-clonal CD4⁺ T cell subsets. Abbreviations: ns: P > .05; *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001; nlm: health donors.

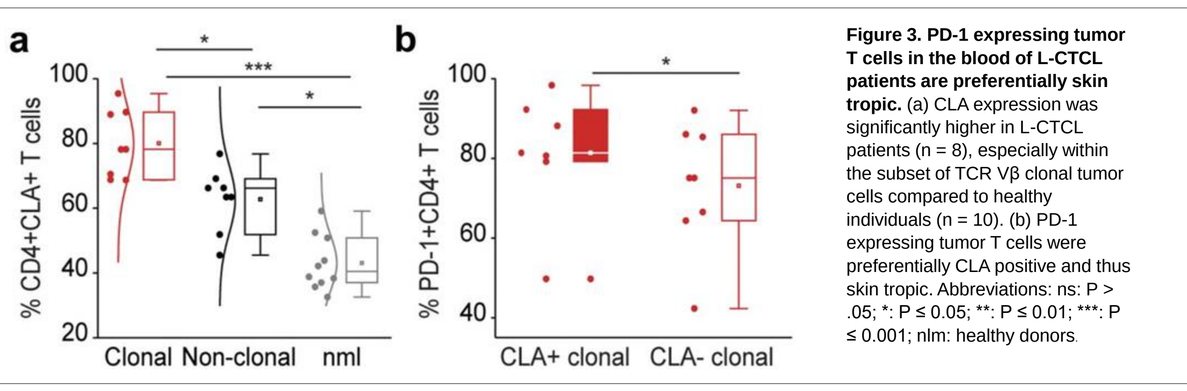

Figure 3. PD-1 expressing tumor T cells in the blood of L-CTCL patients are preferentially skin tropic. (a) CLA expression was significantly higher in L-CTCL patients (n = 8), especially within the subset of TCR Vβ clonal tumor cells compared to healthy individuals (n = 10). (b) PD-1 expressing tumor T cells were preferentially CLA positive and thus skin tropic. Abbreviations: ns: P > .05; *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001; nlm: healthy donors.

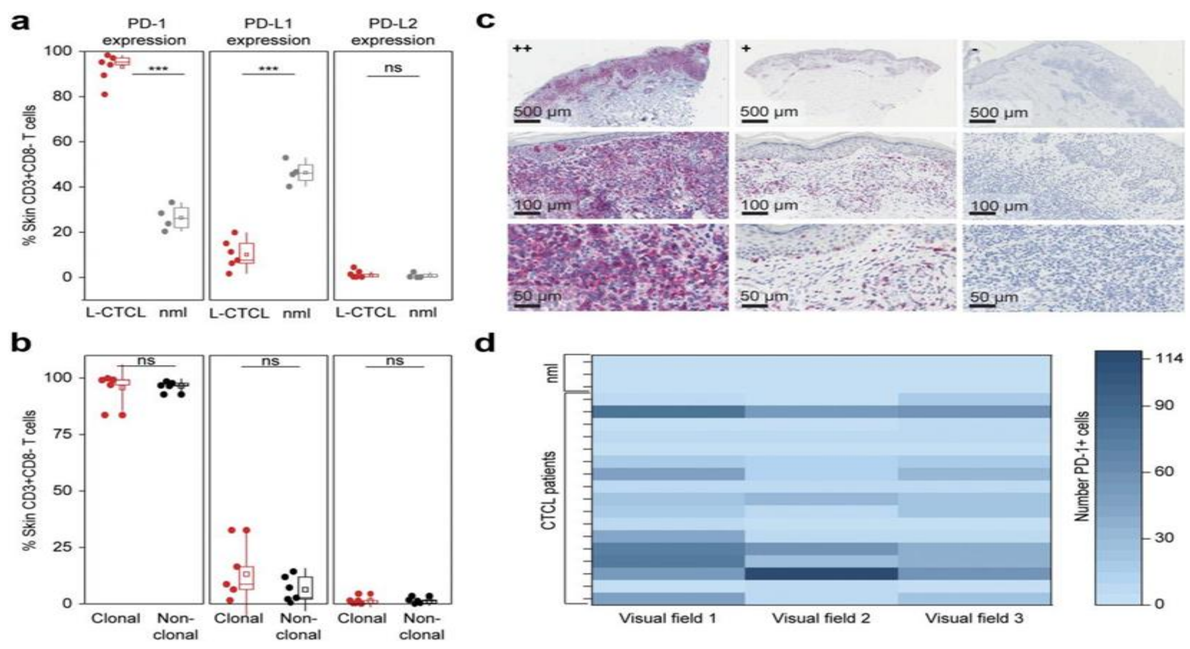

Figure 4. PD-1 is significantly overexpressed, PD-L1 significantly decreased and PD-L2 is low on T cells in the skin of patients with L-CTCL when compared with T cell population in healthy individuals. (a) PD-1, PD-L1 and PD-L2 expression analyzed by flow cytometry in skin-derived CD3+CD8− T cells isolated from biopsies of L-CTCL patients (n = 6) and healthy individuals (n = 4) using short-term explant technique. (b) Expression of PD-1, PD-L1 and PD-L2 in the tumor (clonal) and non-tumoral bystander (non-clonal) T cells from L-CTLC skin lesions. In at least one of the samples analyzed in a) and b), there was a partial aberrant loss of CD4 on the tumor cells. For consistency throughout all the samples, a gating strategy on CD3+ CD8-T cells was applied. (c) Representative images of paraffin-embedded CTCL (MF and SS) skin biopsies with high expression of PD-1 (++/≥50%), with moderate expression of PD-1 (+/5-50%), and negative for PD-1 (-/<5%). (d) Graphic visualization of PD-1 expression in CTCL skin lesions (n = 18). Evaluation of three visual fields .

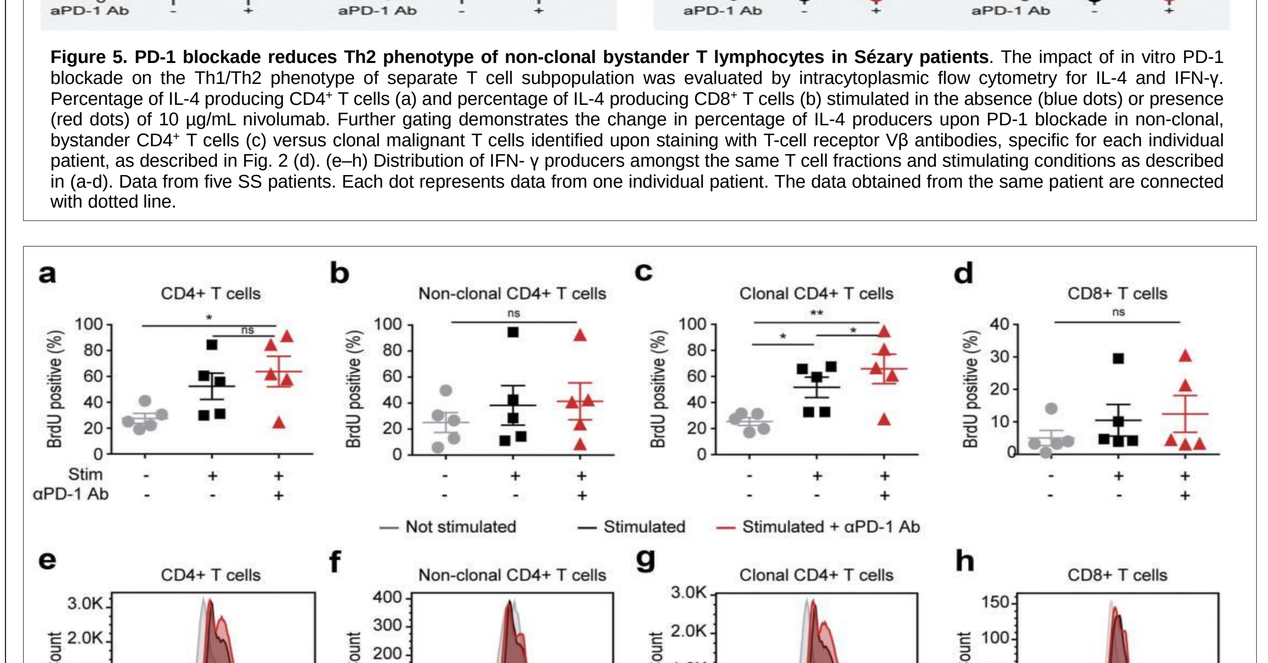

Figure 5. PD-1 blockade reduces Th2 phenotype of non-clonal bystander T lymphocytes in Sézary patients. The impact of in vitro PD-1 blockade on the Th1/Th2 phenotype of separate T cell subpopulation was evaluated by intracytoplasmic flow cytometry for IL-4 and IFN-γ. Percentage of IL-4 producing CD4⁺ T cells (a) and percentage of IL-4 producing CD8⁺ T cells (b) stimulated in the absence (blue dots) or presence (red dots) of 10 µg/mL nivolumab. Further gating demonstrates the change in percentage of IL-4 producers upon PD-1 blockade in non-clonal, bystander CD4⁺ T cells (c) versus clonal malignant T cells identified upon staining with T-cell receptor Vβ antibodies, specific for each individual patient, as described in Fig. 2 (d). (e–h) Distribution of IFN- γ producers amongst the same T cell fractions and stimulating conditions as described in (a-d). Data from five SS patients. Each dot represents data from one individual patient. The data obtained from the same patient are connected with dotted line.

Figure 6. In vitro PD-1 blockade leads to enhanced proliferation of T cells, but affects most strongly the clonal tumor T cells in blood from SS patients. In vitro treatment with nivolumab 10 µg/mL and subsequent detection of cell proliferation upon stimulation with PMA/ionomycin in total CD4+ T cells (a), non-clonal bystander T cells (b), clonal tumor T cells (c) or CD8+ T cells (d). Cell proliferation was assessed by flow cytometry via measurement of the incorporation of non-radioactive 5-bromo-2'-deoxyuridine (BrdU) in newly synthesized DNA. (e-f) Representative histograms depicting how PD-1 blocked affects BrdU incorporation in the different T cell fractions, as described in (a-d). Data are from five individual SS patients. Abbreviations: ns: P > .05; *: P ≤ 0.05; **: P ≤ 0.01

Discussion

We demonstrate that PD-1 expression is markedly increased on tumor T cells compared to non-tumor CD4+ T cells from SS patients and to CD4+ cells from healthy individuals. In contrast, PD-L1 shows decreased expression on tumor T cells, while PD-L2 expression is low without significant differences between these groups. Functional PD-1 blockade in vitro resulted in reduced Th2 phenotype of non-tumor T lymphocytes but enhanced the proliferation of tumor T cells from SS patients. Our study sheds some light on the PD-1 axis in both peripheral blood and skin compartments in SS patients, which may be relevant for the treatment of L-CTCL with immune checkpoint inhibitor.

***Contributed equally.**

1 Saulite I. et al., Oncoimmunology 2020

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Zurich (KEK-ZH-Nr. 2015–0209).