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Immunoistochemical expression of PD-1 and PD-L1 in bone marrow biopsies of patients with acute myeloid leukemia.

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Author's contributions:
Francesco Romano conceived, designed and performed the experiment, analyzed the data and wrote the manuscript.
Antonino Giulio Giannone designed the experiment, analyzed the data and designed the figures.
Sergio Siragusa wrote and revised the manuscript.
Rossana Porcasi performed the experiment and analyzed the data.
Ada Maria Florena provided the samples, wrote and revised the manuscript.

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Background.

Haematological and non-haematological malignancies are able to escape the host immune by the capacity to hijack the immune check-points. Several immune check-point molecules are known, such as T cell immunoglobulin mucin-3 (TIM-3), cytotoxic T-cell antigen-4 (CTLA-4), programmed death-1 (PD-1) with its ligand PD-L1 and others.[1] The function of these immune check-points is to prevent the damage resulting from an excessive activation of the immune response in the setting of chronic antigenic stimulation, thus leading to autoimmune phenomena, as proved in knock-out mice models. PD-1 is normally present on activated T lymphocytes membrane, acting as a negative costimulatory receptor.

PD-L1 is constitutively expressed at low levels by resting lymphocytes, antigen presenting cells and certain immunologically privileged tissues like placenta and testis.

PD-L1 expression can be induced as well. In an inflammatory/infective context when T cells recognize antigens expressed by MHC-complex they start to produce inflammatory cytokines. The resulting inflammation leads to the expression of PD-L1 by hematopoietic, epithelial and endothelial cells, activating PD-1 on the surface of T-cells and therefore blocking the immune response.

Previous studies have found out that PD-1 is highly expressed on T-reg cells and their binding with PD-L1 enhances suppressor T-reg functions[2,3]; the activation of PD-1/PD-L1 pathway reduces the lytic capacity of NK cells and B cell antibody production.

In solid neoplasms PD-L1 expression by cancer cells and persistent up-regulation of PD-1 by tumour-infiltrating lymphocytes is common[45]. All these findings brought to the development of check-point inhibitors in the contest of solid tumors and lymphoproliferative neoplasms such as lymphoma and myeloma where the immune checkpoint blockade treatment have shown efficacy in refractory/relapsed neoplasms[6]. Few investigations only have been conducted on the role of PD-1/PD-L1 in myeloid neoplasms, such as acute myeloid leukemia, a haematological cancer characterised by high-risk of relapse and poor prognosis.

In leukemia, the bone marrow serves as a sanctuary for neoplastic cells, these cells interact with the tumour microenvironment (TME), constituted by stromal cells, endothelial cells and immune cells. The marked activation of the PD-1/PD-L1 pathway contributes to the maintenance of an immunosuppressive microenvironment. In fact, blasts are able, through the production of immunoinhibitory factors, to suppress the function of immunosurveillance and immuno-elimination of the tumor by the effector T cells. The effector T cells are "exhausted" in their capacity to secrete granzyme B, perforine and interferon gamma, and there is an upregulation of the T-reg functions, in addition to the presence of myeloid-derived suppressor cells[7].

PD-L1 expression and his link with PD-1 on activated lymphocytes results in an impaired antitumoral activity in murine models[8]. Zhang et al. investigated the role of PD-1/PD-L1 engagement in murine AML showing that PD-1 -/- mice generated augmented antitumoral response in comparison with wild type mice. Similar results were obtained using anti-PD-L1 antibodies.[9]

A study from Zhou et al. reported how the function of adoptively transferred AML-reactive CTLs was reduced by AML-associated Tregs and how Treg depletion followed by PD-1/PD-L1 blockade showed efficacy for AML eradication in murine models.[10]

Objective of the study.

To assess the presence of PD-1 and PD-L1 positive cells, by immunohistochemistry, in bone marrow biopsies of patients with AML.

Material and methods.

Four micron thickness sections were obtained from the formalin-fixed paraffin-embedded specimens. Haematoxylin-eosin staining was performed to assess the morphologic features of bone marrow. Immunohistochemical stainings were performed using a Ventana Benchmark Ultra automated staining instrument according to the manufacturer's recommendations, using anti-PD-1 (clone

NAT105, Ventana) and anti-PD-L1 (clone 22C3, Dako) antibodies.

Results.

We obtained 34 bone marrow trephine biopsies from newly diagnosed AML patients, 17 males and 17 females with a 67.3 mean age. We used 10 healthy bone marrow specimens as normal controls. None out of 10 control bone marrows resulted positive for either PD-1 or PD-L1 expressing cells as expected. Eleven out of 34 AML bone marrows (32,4%) showed at least 1% of PD-L1 positive cells (fig.2 b,d,f), while 6 AML bone marrow samples (17,6%) were positive for at least 1% of PD-1+ cells (fig. 1 b).

Discussion.

Despite the presence of relevant preclinical data regarding the role of immune check-points, few studies to evaluate the PD1/PDL1 axis have been conducted in myeloid neoplasm. Jia et al. performed flow cytometry analysis on PBMCs and BMMCs of 22 newly diagnosed AML patients and observed a significantly increased frequency of PD-1 expressing CD8 T cells in bone marrow compared to peripheral blood, suggesting a more exhausted status of these cells in relation to the suppressive environment[11]. Dail et al. measured PD-L1 expression in 7 AML patients using immunohistochemistry and flow cytometry and found that PD-L1 was detectable (>2% cells) in all patients.[12] Yang et al. assessed 45 bone marrow biopsies of MDS, CMML and AML patients and found that leukemic blasts of 9 patients (20%) were PD-L1+, while 3 (7%) were positive for PD-1. All 4 controls tested were negative for both PD-1 and PD-L1.[13] Daver et al. measured PD-1 expression on bone marrow aspirates of 74 AML patients using flow cytometry. The results showed higher PD-1 expression compared to healthy controls (n=8).[14]

To our this is the largest study evaluating by immunohistochemistry the expression of PD-1 and PD-L1 in AML bone marrows and shows a significative positivity of the activation of the PD-1/PD-L1 pathway which gives a rationale to further studies regarding the charateristics of the cells involved in the PD-1/PD-L1 pathway and the immunosuppressive microenvironment.

An implementation of the PD-1/PD-L1 pathway evaluation in clinical setting could have prognostic significance since the expression of PD-L1 by AML blasts has been associated with poor-risk and intermediate-risk AML[15], furthermore immune checkpoint inhibitors have shown promising results in maintenance treatment of high-risk AML[16], underlining not only a prognostic but also therapeutic value of the PD1/PD-L1 evaluation.

Figure 1 – PD-1 expression
Acute myeloid leukemia evolved from chronic myeloid leukemia (a); presence of approximately 20% of CD34 + myeloid blasts (b); PD-1 expression in about 4% of myeloid blasts (c).
a: hematoxylin-eosin staining, 200x;
b: CD34 immunohistochemical staining, 200x;
c: PD-1 immunohistochemical staining, 200x.

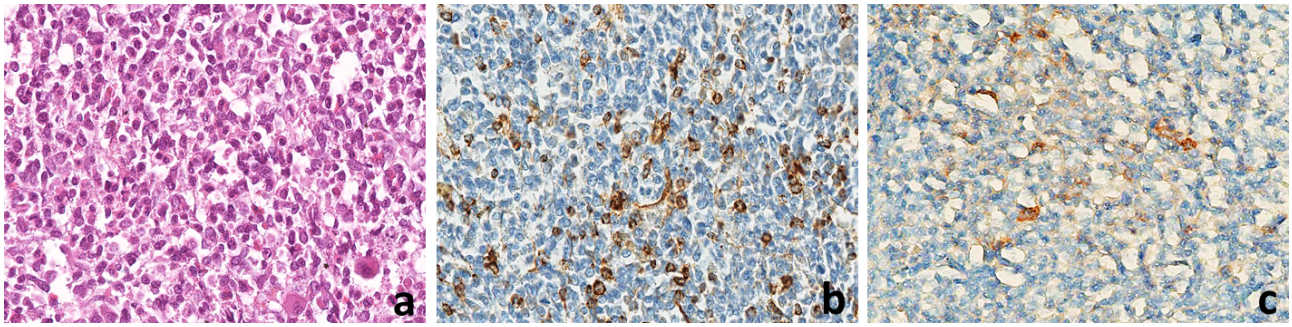
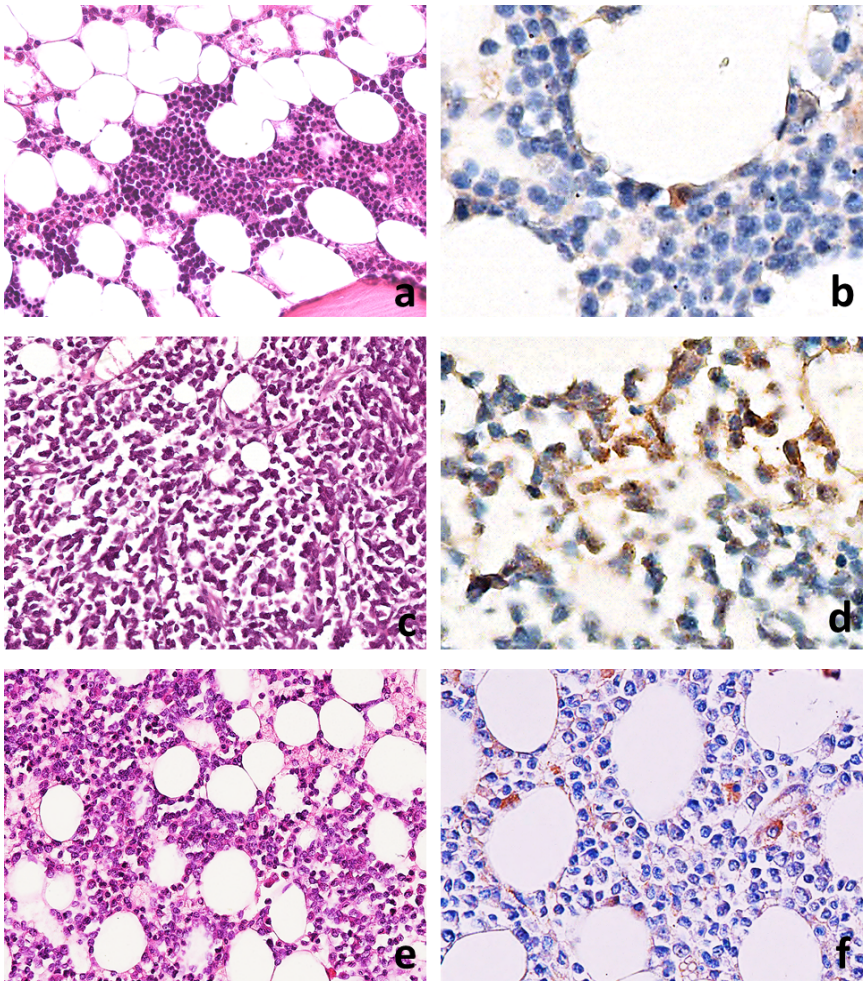


Figure 2 – PD-L1 expression
(a-b) Acute myeloid leukemia with myelodysplastic changes, with marrow infiltration by a blast population CD34 / MPO; Focal expression of PD-L1 in a myeloid blast (b).
(c-d) Acute myeloblastic leukemia with submassive medullary infiltration (blasts MPO+/CD34+ = 85%); PD-L1 + expression in 6% of myeloid blasts (d).
(e-f) Acute myeloid leukemia (blasts MPO+/CD34+ = 30%); PD-L1 expression in 2% of myeloid blasts (f).
a,c,e: hematoxylin-eosin staining, 200x; b,d,f: PD-L1 immunohistochemical staining, 400x.



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