Flow-cytometry and functional evaluation of the CD39/CD73 adenosinergic immunosuppressive axis in patients with Sézary syndrome

Flow-cytometry and functional evaluation of the CD39/CD73 adenosinergic immunosuppressive axis in patients with Sézary Syndrome.

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- Patients with *Sézary syndrome* experience profound immunosuppression……

- Emerging as relatively new immune checkpoint is the production and accumulation of immunosuppressive metabolites in the tumor microenvironment, with adenosine (ADO) as a pivotal example.

- ADO can impair anti-tumor immunity, through the attenuation of protective effector cells, including T and NK cells, and by enhancing the suppressive capacity of T regulatory cells.

Allard B, Allard D, Buisseret L, Stagg J. The adenosine pathway in immuno-oncology. Nat Rev Clin Oncol. 2020;17(10):611-629.
Allard B. Beavis PA, Darcy PK , Stagg J. Immunosuppressive activities of adenosine in cancer. Curr Opin Pharmacol 2016; 29:7-16
Yegutki, G.G. Enzymes involved in metabolism of extracellular nucleotides and nucleosides: functional implications and measurement of activities. Crit. Rev. Biochem. Mol. Biol. 2014;49(6):473-97

Adenosine – a critical checkpoint in the tumor microenvironment

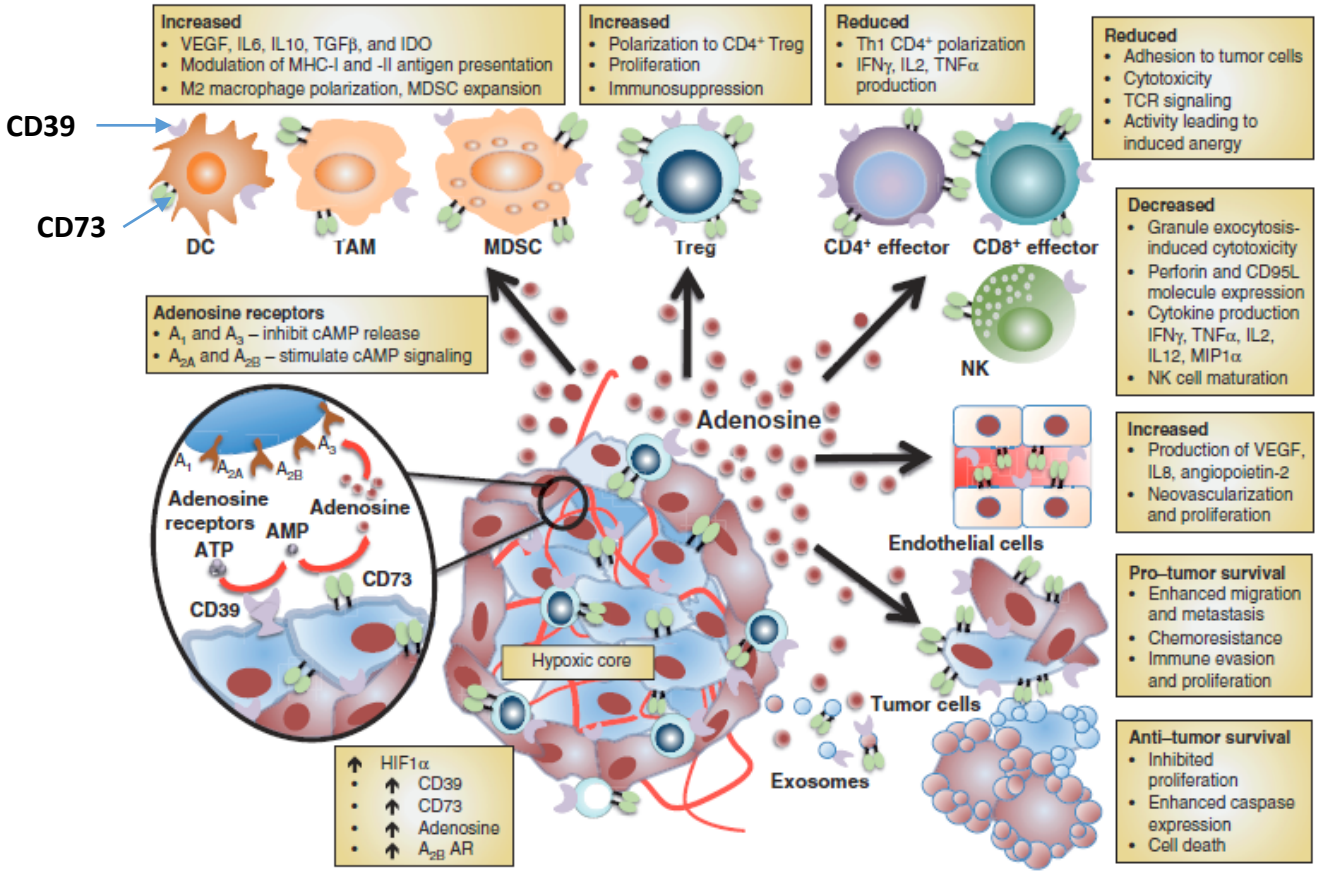
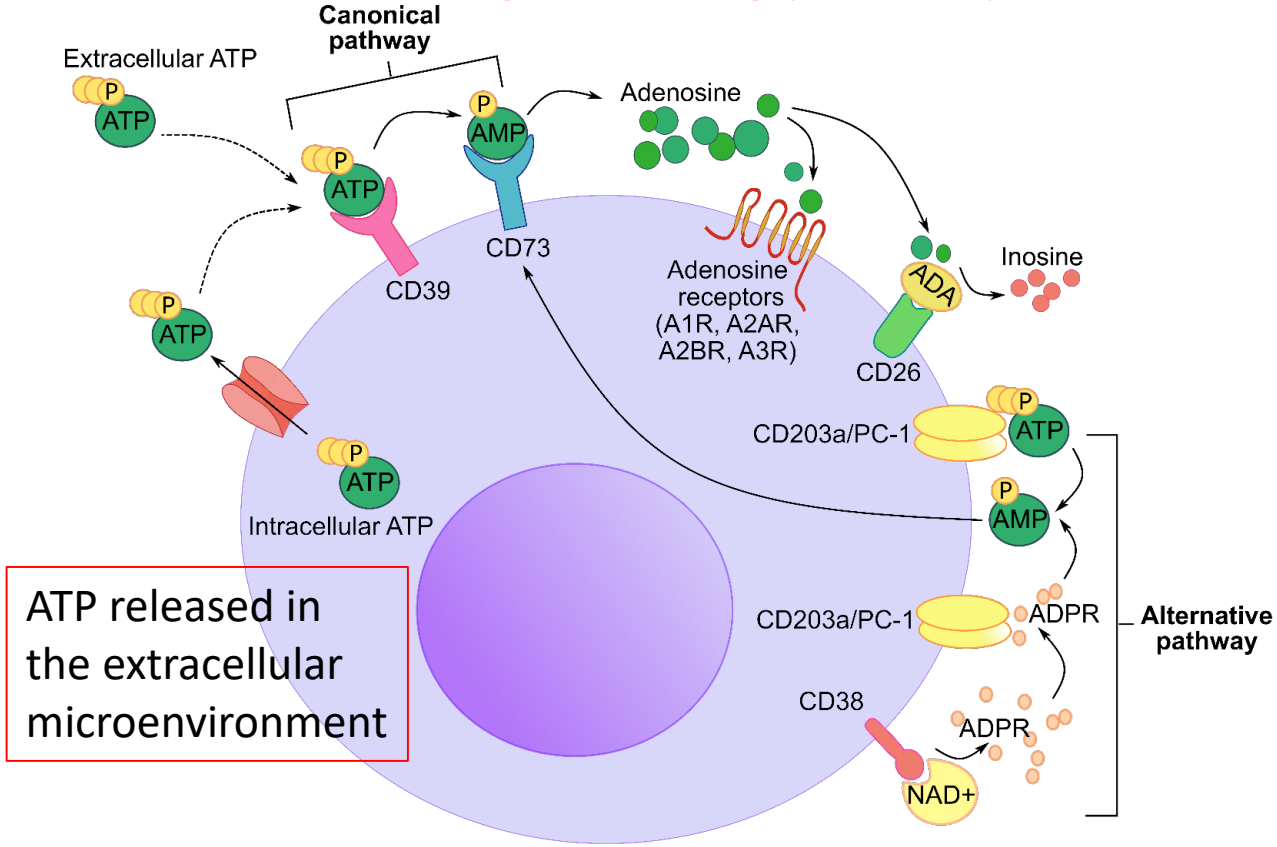


Figure 1. Adenosine-mediated effects in the hypoxic tumor microenvironment. Release of extracellular ATP in the hypoxic tumor microenvironment is converted to adenosine by CD39 and CD73 ectonucleotidases. CD39 and CD73 are broadly expressed across a number of cell types. Modulation of their distribution can vary dependent on cellular activation and tissue localization. Adenosine enhances polarization of myeloid and T-cell subsets to proangiogenic and immunosuppressive phenotypes, enhancing tumor growth and survival. High adenosine levels affect effector immune cells, NK cells, and CD8+ T cells, responsible for cytotoxic killing of aberrant malignancies due to inhibited expression of molecules that mediate cell death. DC, dendritic cells; IDO, indoleamine 2,3 dioxygenase; TCR, T-cell receptor.

Arabella Young, Deepak Mittal, John Stagg, *et al.* Cancer Discovery; 2014, 4: 879

Canonical (CD39 and CD73) and non-canonical (CD38, CD203a, CD73) adenosine-generating pathways

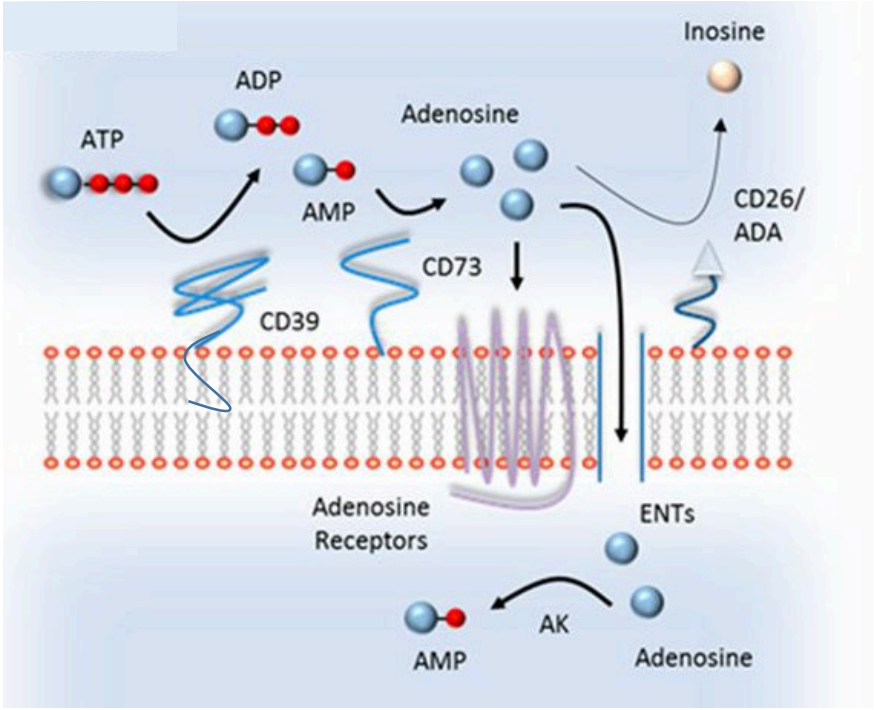


CD39 and CD73 nucleotide-metabolizing enzymes are involved in the adenosine-generating pathway: CD39 cleaves ATP and ADP down into AMP, which is converted into adenosine by CD73.
The extracellular adenosine deaminase/CD26 complex catalyzes the deamination of adenosine to inosine, thus reducing the adenosine levels.

Yegutki, G.G. Enzymes involved in metabolism of extracellular nucleotides and nucleosides: functional implications and measurement of activities. Crit. Rev. Biochem. Mol. Biol. 2014;49(6):473-97

Ferretti E. Horenstein AL, Canzonetta C, Costa F, Morandi F. Canonical and non-canonical adenosinergic pathways. Immunology lett. 2019; 205:25-30

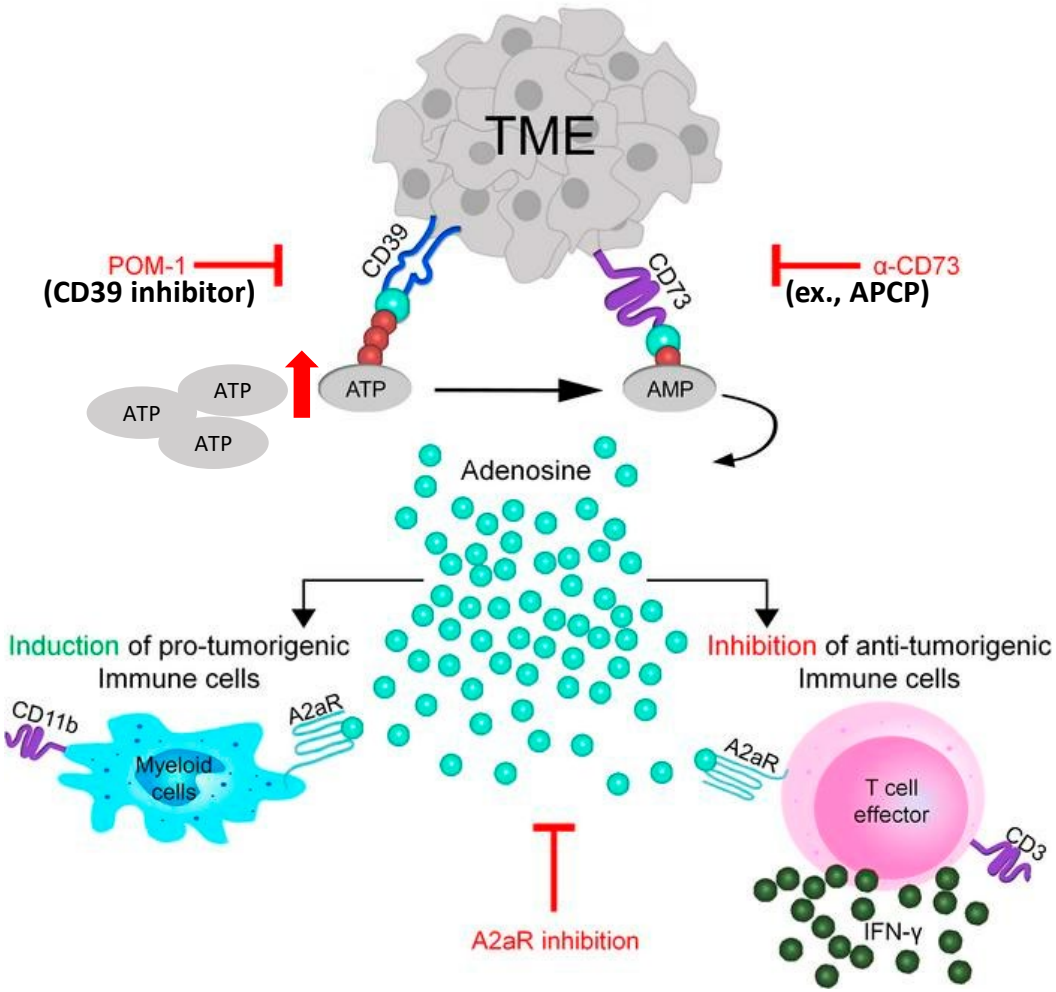
Canonical (CD39 and CD73) adenosine-generating pathway in physiological conditions



Jessica L. Bowser et al. J Immunol 2018;200:897-907 (modified)

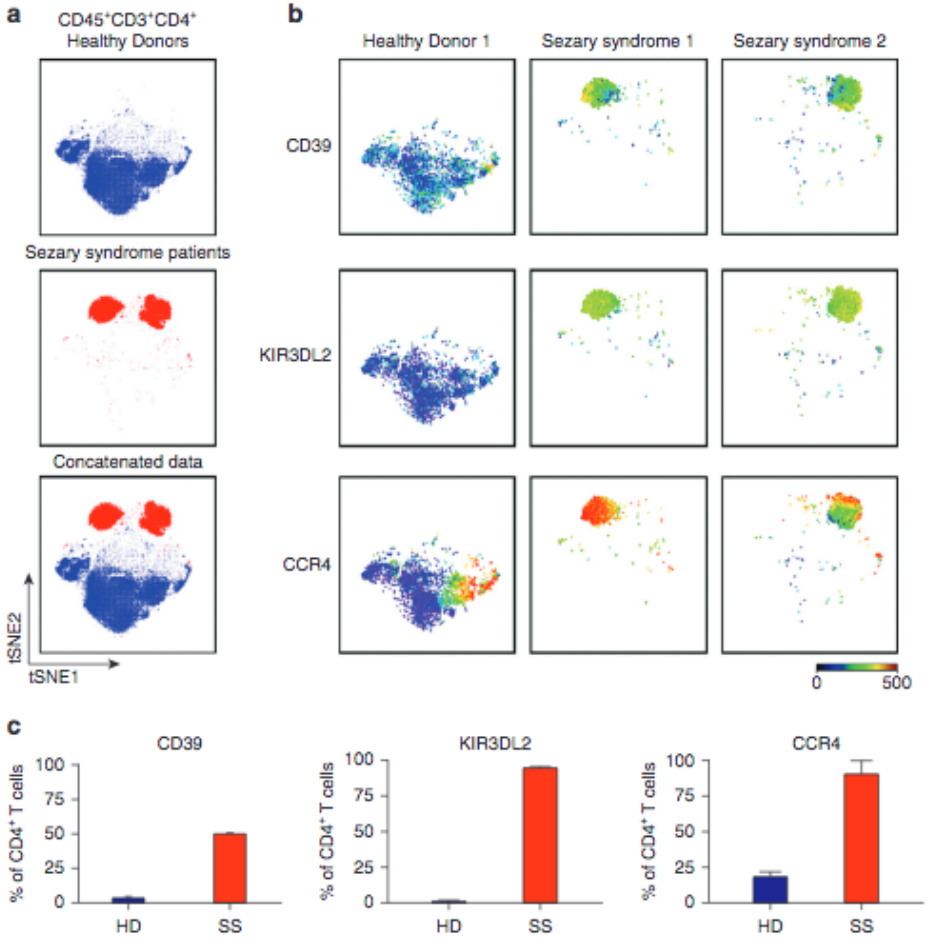
In physiological conditions, the concentration of adenine nucleosides (ATP, ADP, and AMP) at the cell surface is low. Extracellular ATP is converted to adenosine by two phosphohydrolysis reactions by cell surface nucleotidases, CD39 and CD73. CD39 converts ATP to ADP/AMP, and CD73 converts AMP to adenosine. Adenosine can activate adenosine receptors (A1R, A2AR, A2BR, and A3R), be transported into the cell via ENTs, or be converted to inosine at the cell surface by CD26-bound ADA (adenosine deaminase).

Canonical (CD39 and CD73) adenosine-generating pathways in cancer



Identification of CD39 as a Marker for the Circulating Malignant T-Cell Clone of Sézary Syndrome Patients

Journal of Investigative Dermatology (2019) **139,** 725–728; doi:10.1016/j.jid.2018.09.026



The objective of this study were:

to investigate the expression of the of CD39 and CD73 nucleotide-metabolizing ectoenzymes in peripheral blood from SS patients;

to define the contribution of the CD39/CD73 adenosinergic immunosuppressive pathway to tumor escape from immune response and immune dysfunctions in patients with SS.

Experimental Design: observational study

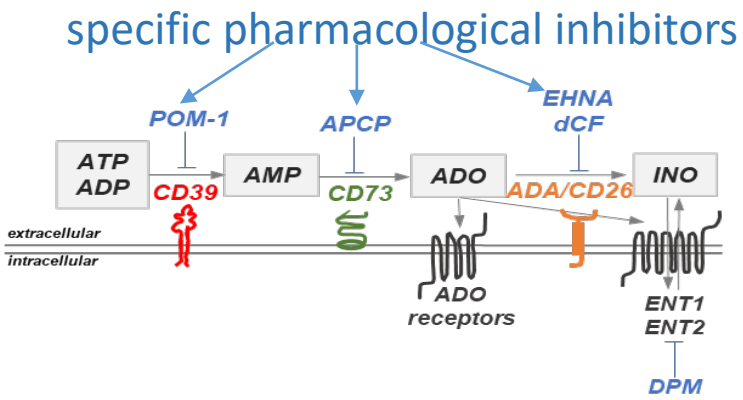
Study population SS patients at diagnosis and patients currently under treatment at the Dermatologic Clinic, Dept Medical Sciences, University of Turin, Italy will be prospectically enrolled (expected number: 10-12 patients)

Immunophenotypic analysis of nucleotide-metabolizing ectoenzymes in peripheral blood from patient with SS

The expression of CD39, CD73, CD38, CD203a/PC-1 and adenosine receptor A2A (A2AR) will be analysed by flow cytometry along with conventional diagnostic and lineage markers (including, CD3, CD4, CD158k, CD8, CD26, CD7, CD25, CD27, CD16, CD14, CD56, CD127 and PD-1) in the neoplastic T clone, CD4+ T cells, CD8+ T cells, B cells, NK cells and Treg cells. Peripheral blood from healthy subjects will be used as control.

Methods: Multiparametric flow cytometry analysis will be carried out in whole blood to define a high-content molecular signature for each cell subpopulation. Flow cytometric analyses will be performed with a FACS Canto (BD Biosciences) cytometer and the raw data generated will be analyzed with FlowJo sotware 10.1 (Tree Star).

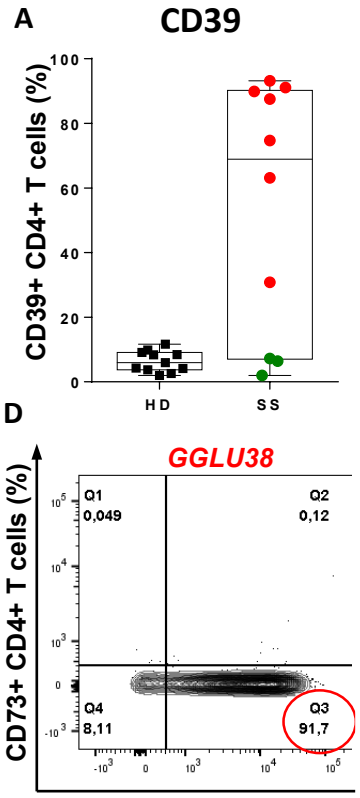
Canonical (CD39 and CD73) adenosine-generating pathways



Nucleotide consumption and ADO production by malignant CD4+ T cells isolated from SS patients

We compared the ability of peripheral blood CD4+T cells from SS patients and HD to hydrolyze ATP and to convert AMP into adenosine, in vitro. Briefly, CD4+ Tcells from SS/HD were seeded in 48-well plates in HBSS, pretreated or not with specific inhibitors for 1h and then incubated with exogenous (e) eATP (patients with high CD39) or eAMP (patients with high CD73) at 37°C. After 1h incubation, analyses of the supernatant were performed with an RP-HPLC.

CD39 or CD73 are overexpressed on CD4+ malignant T cells from SS patients

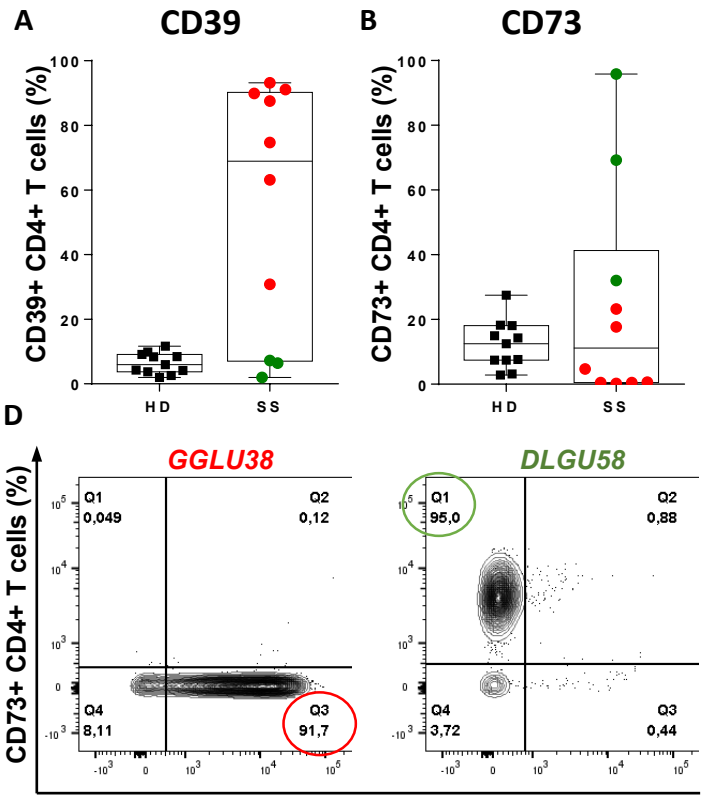


7 out of 10 patients had high CD39

One representative SS patient with high CD39 expression is shown

Two subgroups of patients can be clearly identified based on the mutually exclusive overexpression of CD39 or CD73 in CD4+ T cells.

CD39 or CD73 are overexpressed on CD4+ malignant T cells from SS patients

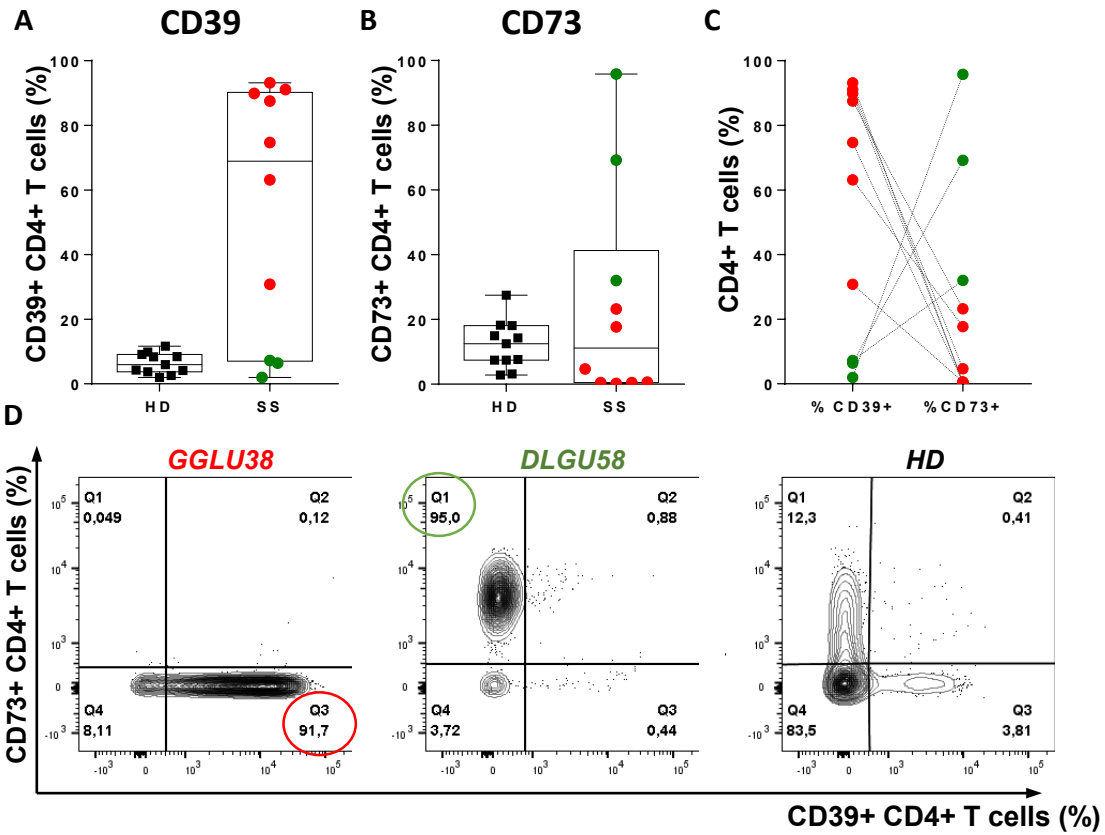


3 out of 10 patients had high CD73

One representative SS patient with high CD73 expression is shown

Two subgroups of patients can be clearly identified based on the mutually exclusive overexpression of CD39 or CD73 in CD4+ T cells.

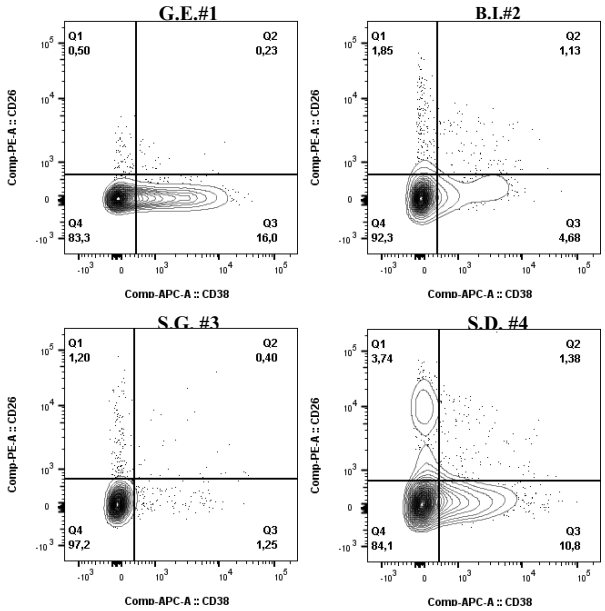
CD39 or CD73 are overexpressed on CD4+ malignant T cells from SS patients



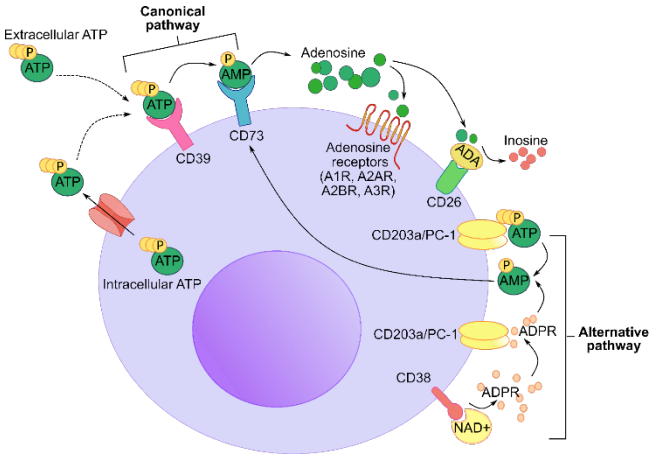
CD39 and CD73 are mutually exclusive

CD4+T cells with high CD39 have low CD73, vice versa those with high CD73 have low CD39

..and what about CD38?

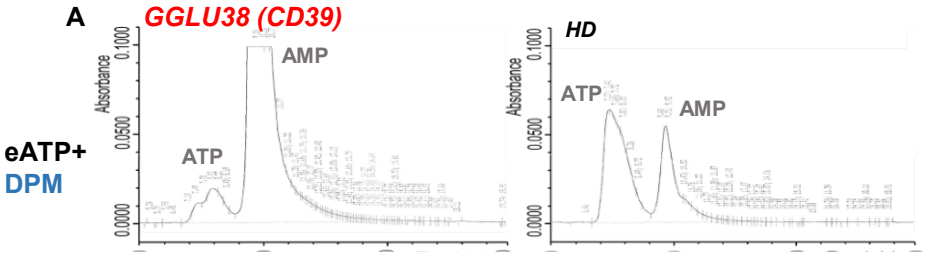


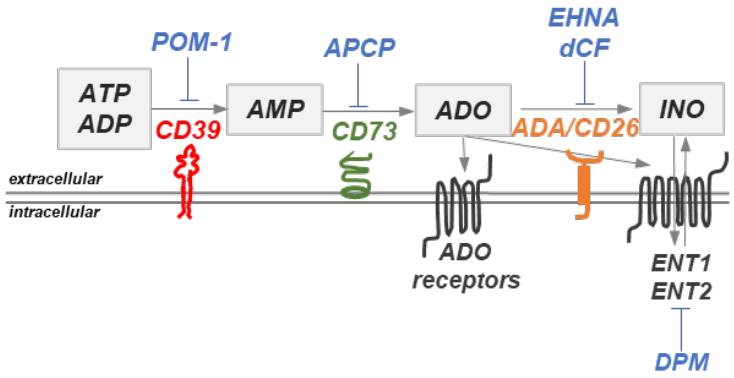
Patients	CD3+CD4+ (%)	CD4+CD26- (%)	CD4+CD26-CD38+ (%)
G.E.#1	88.6	99.3	16
B.I. #2	89.8	96.98	4.68
S.G.#3	89.5	98.45	1.25
S.D.#4	66.3	94.9	10.8



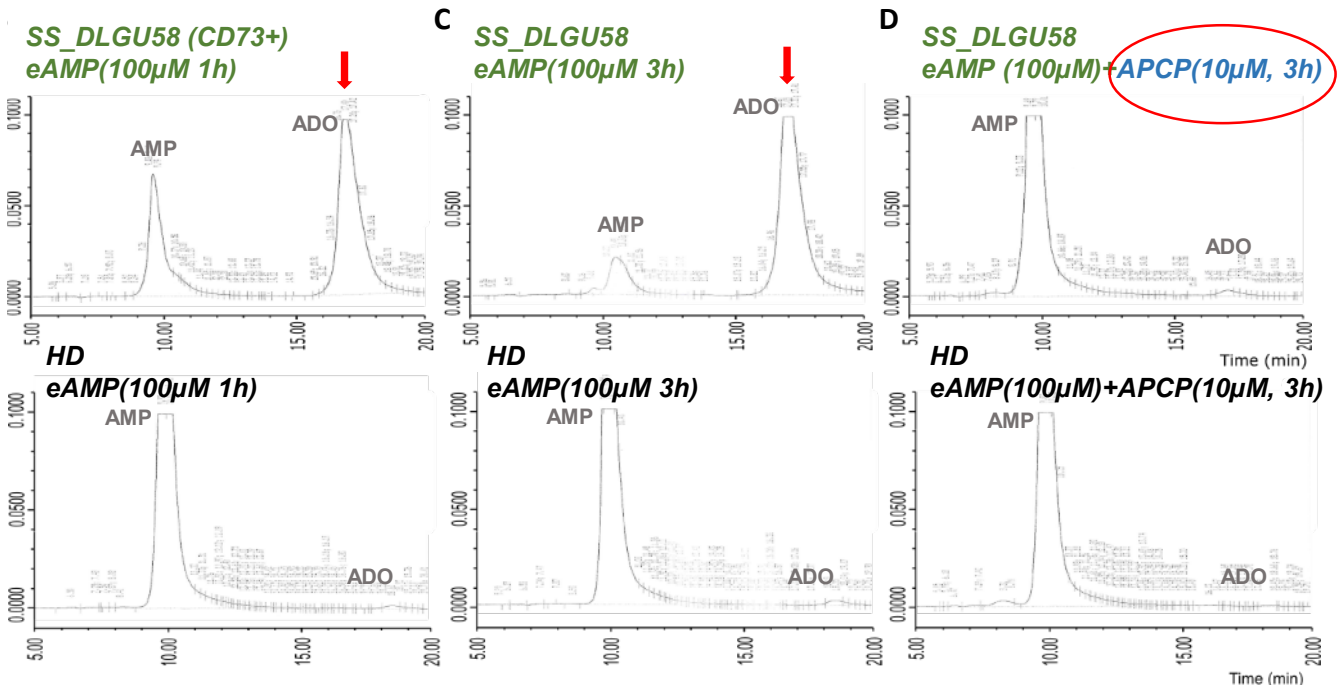
CD39 and CD73 expressed by malignant CD4+ T cells are biologically active

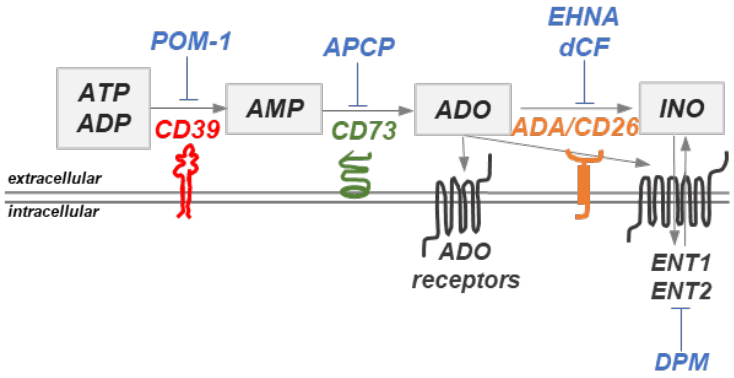
supernatants of CD4+ T cells from SS patients after 1h exposure to the indicated compounds (eATP)





CD4+ T cells from SS patients with high levels of CD39 showed an increased ability to hydrolyze ATP with increased generation of AMP compared to normal control cells.





In parallel, CD4+ T cells with high levels of CD73 showed increased conversion of AMP into ADO, respect to normal control cells.

CONCLUSIONS

The aberrant expression of CD39 and CD73 along with loss of CD26 expression in circulating Sézary cells suggest that the sequential activity of CD39 and CD73 ectoenzymes scavenges ATP and generates immunosuppressive adenosine in the tumor microenvironment contributing to tumor immune escape.

The results inferred from this study are the starting point for more comprehensive studies towards the development of new therapies targeting the CD39/CD73 adenosinergic axis in order to overcome tumor immunosuppression, allowing the induction of effective anti-tumor immune response.

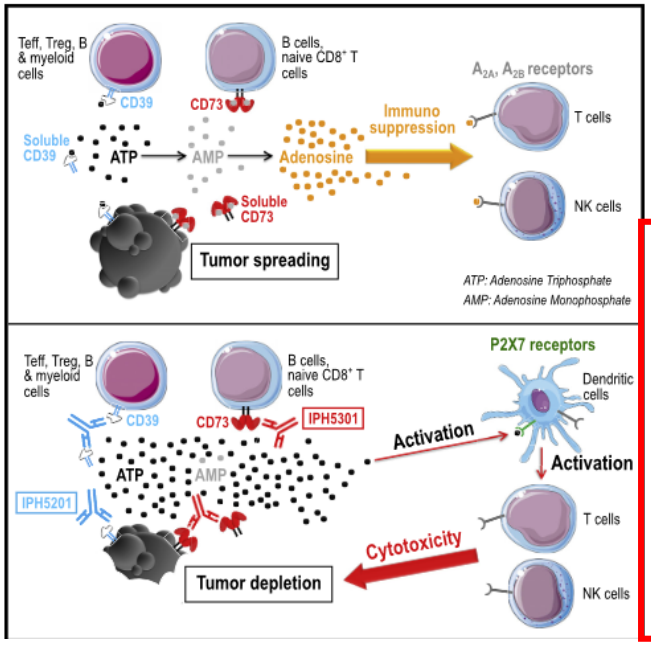
CD39: specific marker for the evaluation of SS patients' circulating tumor burden but also as a promising target in the context of SS with the development of CD39/CD73-blocking antibodies that may restore efficient antitumor responses.

Article

Cell Reports

Blocking Antibodies Targeting the CD39/CD73 Immunosuppressive Pathway Unleash Immune Responses in Combination Cancer Therapies

Graphical Abstract



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In Brief

The production of adenosine via CD39 and CD73 ectoenzymes participates in an immunosuppressive tumor microenvironment. Perrot et al. generated two antibodies, IPH5201 and IPH5301, targeting human CD39 and CD73, respectively. *In vitro* and *in vivo* data support the use of anti-CD39 and anti-CD73 mAbs in combination cancer therapies.

J Invest Dermatol. 2019 Mar;139(3):725-728.
Identification of CD39 as a Marker for the Circulating Malignant T-Cell Clone of Sézary Syndrome Patients
Armand Bensussan, Baptiste Janela, Nicolas Thonnart, Martine Bagot, Philippe Musette, Florent Ginhoux, Anne Marie-Cardine

Perrot et al., 2019, Cell Reports 27, 2411–2425
May 21, 2019
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Future Plans

- Immunophenotypic and immunohistochemical analysis of CD39 and CD73 nucleotide-metabolizing ectoenzymes and adenosine receptor A2A in peripheral blood and skin biopsies from patients with SS

- Correlation with the clinical features of patients at baseline, and with clinical response (complete plus partial versus stable disease or progression), clinical benefit (complete + partial response + stable disease versus progression), response duration, and development of infectious complications

- Functional analysis of the contribution of the CD39/CD73 adenosinergic immunosuppressive pathway to tumor escape from immune response and immune dysfunctions in patients with SS (CD8+ T and NK cells)

- Evaluation of the ability of specific inhibitors to block CD39/CD73 adenosine-signaling in order to restore the antitumor immune response and revert immunosuppression.

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