CD69 as a prognostic marker in Chronic lymphocytic leukemia

Amal Ahmed Zidan[1], Ayman Fathy Arafa[2], Mai Mohamed Shawky[1], Nahla Ibrahim Zidan[1], Salah Al-Sayed Ibrahim Ali [1],

Clinical Pathology Department, Faculty of Medicine, Zagazig University, Egypt
Hematology Department, Faculty of Medicine, Zagazig University, Egypt
Corresponding author: Mai Mohamed Shawky
Email: Maishawky@gmail.com

Article History: Received: 21.06.2023 **Revised:**04.07.2023 **Accepted:** 16.07.2023

Abstract:

Chronic lymphocytic leukemia is very heterogeneous disease with some patients experiencing rapid disease progression. Therefore, it is mandatory to find single and new parameter like CD69 in order to address its prognostic significance with standard prognostic marker in CLL.

Keywords: CD69, Chronic lymphocytic leukemia, T cells.

DOI: 10.53555/ecb/2023.12.1144

I. Introduction

The CD69 gene is located within the natural killer (NK) gene complex on mouse chromosome 6 and human chromosome 12 and codes for a type II transmembrane glycoprotein with a C-type lectin domain, is known to be a marker of early leukocyte activation ***(1, 2).*** CD69 is also known to be expressed on tissue-resident memory T (TRM) cells and to play an important role in the residency of these cells within various tissues ***(3, 4).***

Furthermore, studies have shown that the expression of CD69 on activated T cells is required for the recruitment and maintenance of these T cells in inflamed tissues, establishing and exacerbating tissue inflammation ***(2,5).*** These data suggest that within various tissues, CD69 plays a crucial role in the retention of the cells on which it is expressed. One well-known mechanism underlying the cell retention mediated by CD69 is Sphingosine-1-phosphate receptor 1 (S1P1)-dependent ***(6)***, in which CD69 inhibits the surface expression of S1P1 via direct interaction and prevents the exit of CD69-expressing cells from the thymus and lymph nodes (LNs) ***(7, 8).*** studies have shown that the CD69 ligands, myosin light chain 9 and 12 (Myl 9/12), which are expressed in inflamed tissues, are used to recruit CD69-expressing activated T cells into inflamed tissues for their retention ***(5)*** (figure 1)***.***

The involvement of CD69 in tumor progression has been examined using CD69 deficient mice and anti-CD69 antibody treatment ***(9, 10)***. The involvement of NK cell-mediated anti-tumor responses ***(10)*** and

dendritic cell-based anti-tumor effects ***(9)*** were suggested. However, the cellular and molecular mechanisms, including the role of CD69+ T cells in anti-tumor immunity and the reason why CD69 deficiency results in enhanced anti-tumor responses, remain unclear. Tumor progression is known to be associated with the exhaustion of tumor-infiltrating T lymphocytes ***(11)***.

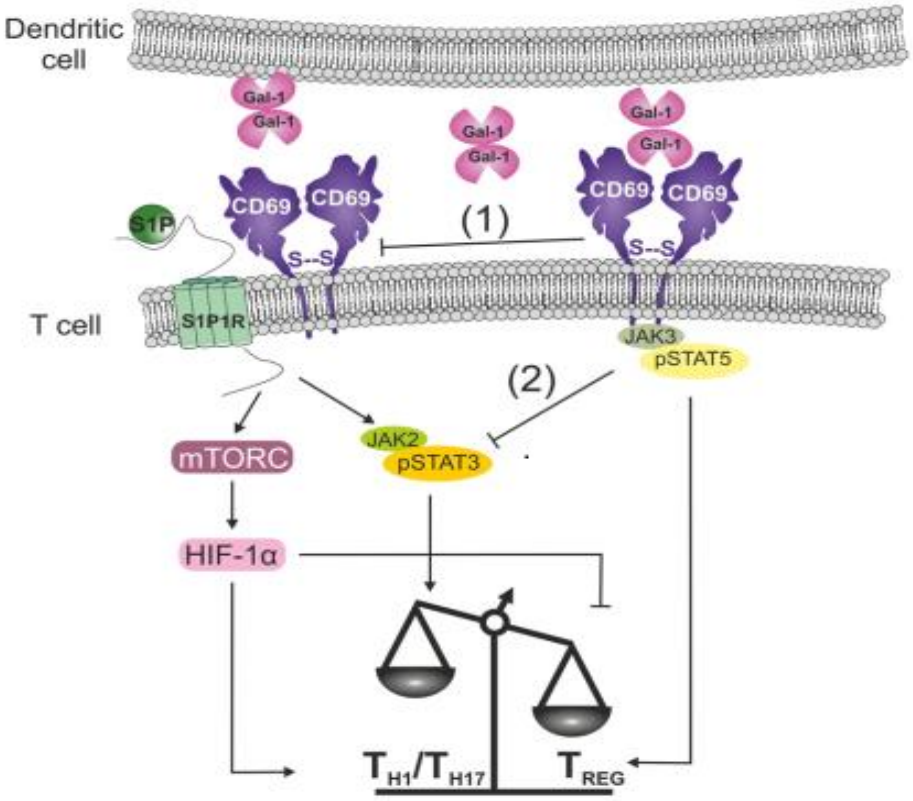

Figure 1. CD69 counteracts S1P1 signaling that favors TH1/TH17 polarization. Picture showing intracellular signaling associated to the expression of S1P1 on the membrane. The binding of S1P to S1P1 receptor increases mTOR/HIF-1α activation as well as increase of JAK2/pSTAT3 pathway. Both signaling routes increase TH1/TH17 effector phenotype and prevent Treg-cell differentiation. CD69 expression in activated lymphocytes prevents S1P1-induced signaling by promoting the internalization and degradation of the receptor and by increasing the JAK3/pSTAT5 pathway, which counteracts STAT3-induced expression of IL-17 and promotes Treg development. The interaction of CD69 with putative ligands, for example Gal-1 (soluble, bound to the plasma membrane of dendritic cells, either directly or through an unidentified, glycosylated, co-receptor) could potentially modulate these CD69-mediated effect ***(1, 2)***.

II.CD69 is an early activation marker

CD69 expression is rapidly induced on the surface of T lymphocytes after TCR/CD3 engagement, activating cytokines and polyclonal, mitogenic stimulation. Transcriptional expression of the CD69 gene is detected early after activation (30-60 min); however, it declines rapidly after 4–6 h. CD69 protein expression can be detected as early as 2–3 h after stimulation. The appearance of CD69 on the plasma membrane of activated cells is faster than that of CD25, underlying its widespread use as a very early marker of lymphocyte activation ***(12).***

CD69 is expressed on infiltrated leukocytes at inflammatory sites in several human chronic inflammatory conditions, for example rheumatoid arthritis**,** systemic lupus erythematosus**,** systemic sclerosis, allergic asthma and atopic dermatitis ***(13)***. Early studies with CD69-deficient cells were unable to definitely prove the function of CD69 in T lymphocyte proliferation and priming ***(14)***. However, in vivo strategies using CD69-deficient mice and blocking antibodies, showed that CD69 expression modulates the severity of different murine inflammation models, including arthritis; asthma and contact hypersensitivity; myocarditis; pathogen clearance; tumor immunity; and inflammatory bowel disease ***(15, 16).***

III. immunoregulatory role of CD69

The in vivo models initially chosen for the study of CD69 function were based on its pattern of expression. Studies in CD69-transgenic mice focused on thymic selection, a process in which CD69 expression is transiently induced. Despite the in vitro evidence suggesting a possible proinflammatory role for CD69, constitutive expression of CD69 by T cells in transgenic mice is not associated with inflammatory conditions ***(17, 18*)**. Furthermore, analysis of antigen-specific responses in mice has not revealed reduced T-cell activation in the absence of CD69**,** suggesting that this receptor does not exert a net positive co-stimulatory effect in T cells in vivo, although a redundant role as a positive co-stimulus for T cells cannot be ruled out ***(19)***.

Given the somewhat contradictory in vitro and in vivo results, it became appropriate to study the role of CD69 in an in vivo model of chronic inflammation. This analysis was based on two lines of evidence. First, CD69 is persistently expressed at inflammatory foci ***(20)***. Second, the CD69 gene is located at the Murine collagen induced arthritis (CIA3) trait loci on rat chromosome 4 and mouse chromosome 6, syntenic to human 12p12–p13, a region that contains susceptibility loci for several autoimmune diseases, including collagen induced arthritis (CIA)**.** Remarkably, the study of CIA in CD69-deficient mice unveiled a new regulatory role for CD69 ***(21, 22)*** (Figure 2).

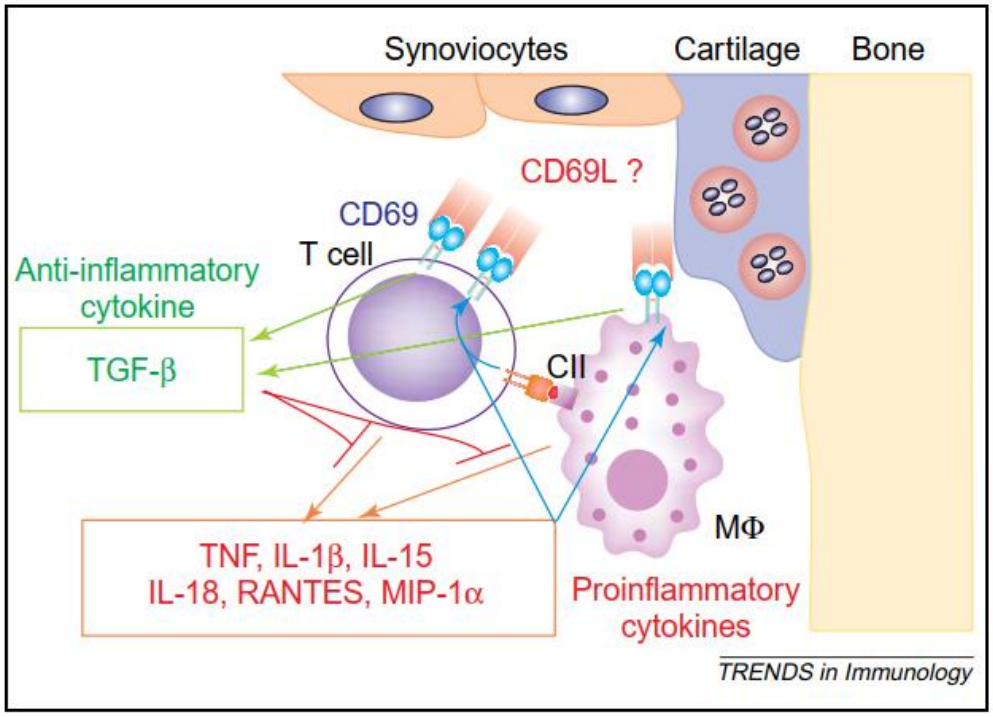

Figure 2. CD69 acts as an immunoregulatory molecule through the production of TGF-b. In collagen-induced arthritis, T cells are activated by collagen-derived peptides (CII) presented by macrophages (Mf), which induce CD69 expression and the release of proinflammatory cytokines. These cytokines and co-stimulatory molecules contribute to the persistent expression of CD69 in the inflammatory foci. Under such circumstances, the interaction of CD69 with its putative ligand(s) (CD69L) would induce the synthesis of the anti-inflammatory cytokine TGF-b, which reduces the secretion of proinflammatory cytokines and the activation of immune cells, thereby ameliorating tissue damage ***(23)***.

CD69-deficient mice develop an exacerbated form of CIA with higher T- and B-cell responses against collagen. This hyper-responsiveness correlates with reduced levels of TGF-b in inflamed joints (Figure 3). Transforming growth factor beta (TGF-b) acts as an anti-inflammatory cytokine in CIA, and treatment with blocking anti-TGF-b antibodies exacerbates arthritis severity, increasing proinflammatory cytokines and chemokines, in wildtype but not in CD69-deficient mice***(23)***. The reduced levels of TGF-b and the absence of CD69 could be causally associated. In this regard, CD69 crosslinking in vitro promotes TGF-b synthesis ***(24).*** In addition, TGF-b synthesis is dependent on ERK activation ***(25)*** and CD69 cross-linking mediates ERK activation ***(26).*** Hence, the regulatory effects of CD69 in vivo appear to be mediated through the synthesis of a pleiotropic cytokine, which might be finely tuned by the controlled expression of CD69 ligand(s) ***(26)***.

CD69 cross-linking induces TGF-b production in CD4C and CD8C T cells as

well as in NK cells and macrophages ***(23)***, suggesting that this receptor exerts a wide immunoregulatory action, and that other cells, expressing the corresponding counter-receptor, might also participate in this phenomenon. Thus, CD69 could influence not only adaptive but also innate immunity. Accordingly, in an NK-sensitive tumour model in mice, CD69 deficiency leads to reduced TGF-b synthesis by immune cells that results in a high production of chemokines, with decreased lymphocyte apoptosis, accumulation of NK cells and enhanced tumor lysis ***(24)***. Supporting these data, blockade of TGF-b signaling in T cells enhances antitumor immunity by facilitating the expansion of tumour-specific CD8+ T cells ***(27).***

Both the NK-sensitive tumor model and the CIA model demonstrates that CD69 deficiency leads to diminished TGF-b levels that support an enhanced immune response, resulting in a more efficient depletion of tumours or increased inflammation in the CIA model. The use of an antibody that downregulates CD69 expression in vivo reproduced in wildtype mice the phenotype found in CD69-deficient mice ***(24)***, further supporting the proposed immunoregulatory role of CD69. However, as mentioned earlier, CD69 cross-linking in vitro also mediates production of proinflammatory mediators, thus suggesting that CD69 could have a dual role, mediating the synthesis of different cytokines, depending on the particular cellular context ***(23)***.

IV.CD69 ligands

The identification of specific ligands for CD69 is critical to understand its roles in physiology and pathology. Based on the identification of a C-type lectin-like domain (CTLD) region within its structure, early studies postulated that CD69 could bind to carbohydrate moieties. However, the results of those early experiments were not conclusive, likely due to the fact that CD69 interacts with both, carbohydrate and protein residues ***(12*)**. These early inquiries planted the seed for a later study in which the galectin-1 protein (protein Gal-1) was identified as a specific ligand of CD69 ***(28)***.

Gal-1 is a carbohydrate-binding protein expressed in dendritic cells and macrophages. Its systemic deficiency exacerbates TH1 and TH17 responses ***(29),*** similar to the phenotype observed in CD69-deficient mice. The binding of CD69 to Gal-1 on dendritic cells (DCs) negatively modulates the in vitro differentiation of TH17 cells ***(28)***, which could control inflammatory responses in vivo. Gal-1 also enhances interleukin-10 (IL-10) secretion in T cells through the activation of the aryl hydrocarbon receptor (AHR) pathway, although the mechanism is not fully elucidated ***(30).***

Another putative ligand of CD69 is the S100A8/S100A9 complex, which binds to CD69 in a glycosylation-dependent manner, and regulates Treg-cell differentiation ***(31).*** In addition to these ligands, which bind CD69 in a trans manner, CD69 establishes lateral (cis) interactions

with the sphingosine 1-phosphate receptor 1 (S1P1) ***(7)***, that regulates lymph nodes T-cell egress, and the amino acid transporter complex LAT1-CD98. These data suggest that, in addition to its possible role as a signal transducer of its ligands, CD69 may independently modulate the function of at least these two receptors, S1P1 and LAT1, which are involved in migration and cell metabolism, respectively ***(32)*** (Figure 3).

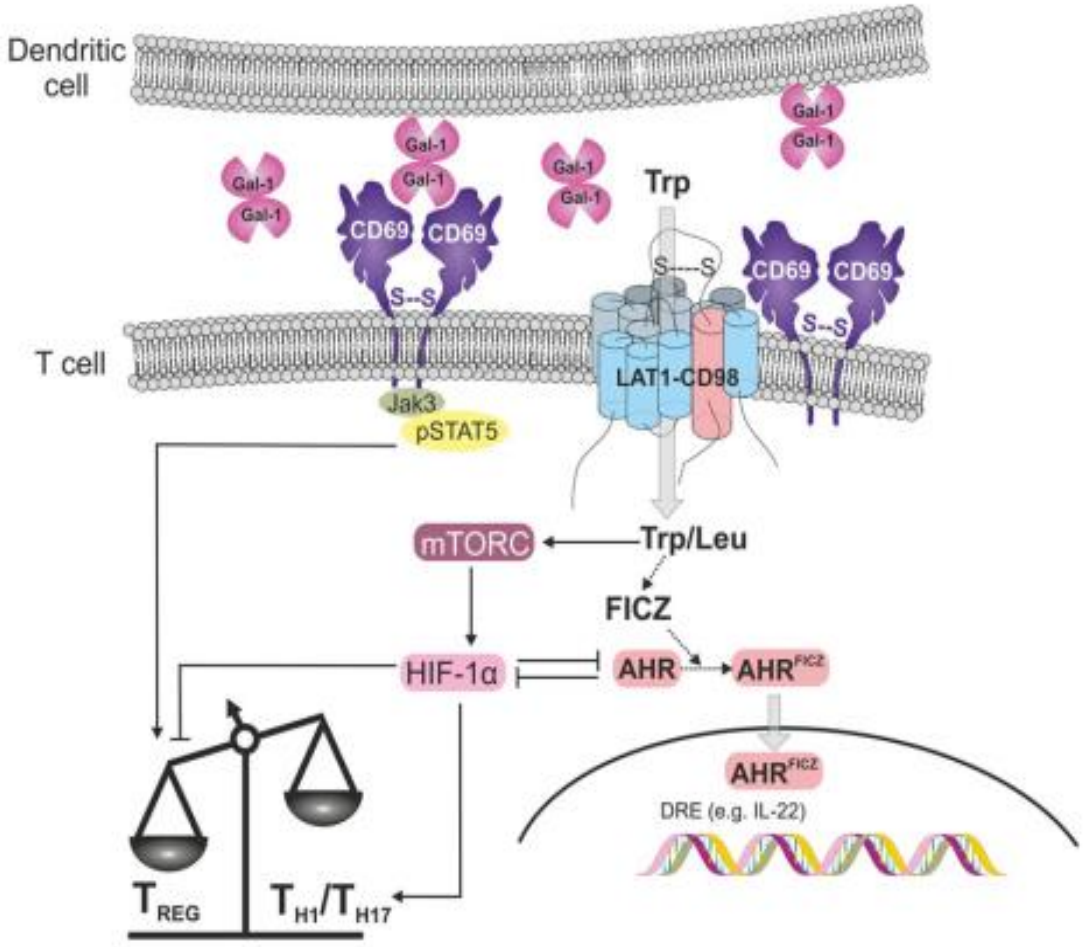

Figure 3. Lateral association of CD69 with the amino acid transporter LAT1-CD98 complex regulates TH1/TH17/Treg balance. Cartoon depicts the lateral interactions of CD69 with the LAT1-CD98 amino acid transporter complex. CD69 increases Trp transport through LAT1-CD98 and favors AHR activation through binding of the Trp-derived FICZ ligand. Activation of AHR-transcriptional activity favors IL-22 secretion. Amino acid uptake through CD69/LAT1-CD98 complex also favors mTOR activation, which promotes TH1 and TH17 development and prevents Treg-cell differentiation. mTOR also controls HIF-1α, which negatively regulates Treg-cell function by promoting Foxp3 degradation. AHR expression attenuates HIF-1α-mediated effects and modulates the TH-phenotype of effector T cells***(32)***.

V.CD69-S1P1 balance in the plasma membrane of T cells determines tissue egress or retention

Immune surveillance and antigen patrolling involves the continuous egress and return of naïve T cells from the lymphoid organs to the circulation. Murine T-cell egress from lymphoid organs is mediated by S1P1 expression and its interaction with sphingosine-1 phosphate (S1P) from the bloodstream. S1P is a sphingolipid metabolite that stimulates diverse signaling pathways, including calcium mobilization, actin polymerization, chemotaxis/migration and survival. S1P can bind to five related G-protein-coupled receptors (S1P1-5) although the S1P1 isoform is the most represented in murine T lymphocytes ***(33).*** Antigen recognition by the T cell receptor (TCR) as well as certain pro-inflammatory stimuli such as interferon (IFN-α/ β) and tumor necrosis factor (TNF-α) can temporarily impair lymphocyte egress from the lymph nodes**.** These stimuli can also directly induce CD69 expression in lymphocytes ***(34)***.

Although the mechanism is not completely established, IFN-AR signaling promotes the formation of a complex that includes CD69 and S1P1 on the plasma membrane. This complex negatively regulates the egress function of S1P1, thus promoting lymphocyte retention in secondary lymphoid organs ***(7)***. This interaction is independent of the CTLD region of CD69 and involves the transmembrane and membrane proximal regions of CD69, and helix 4 of S1P1. CD69 and S1P1 association can be detected by co immunoprecipitation, whereas no association is detected between CD69 and the related receptor S1P3. Increased CD69 expression on the surface of activated T cells promotes S1P1 receptor internalization and degradation with no effect at a transcriptional level ***(8)***.

These biochemical studies placed CD69 as a regulator of lymphocyte migration and promoter of T-cell retention in the lymph nodes and throughout the body. Upon activation, T lymphocytes express CD69 and are transiently retained in the lymph nodes, likely to favor their full activation. For this reason, CD69+ T cells are seldom found in the circulation compared to lymph nodes or inflamed tissues, although they appear increased in some chronic inflammatory conditions ***(35)***. In addition, CD69 is required for the trafficking of effector CD4+ T cells to the bone marrow, particularly for their relocation and the persistence of their interaction with stromal cells as memory T helper cells ***(36).*** A role for CD69 regulation of migration through the control of S1P1 expression has been also reported in skin dendritic cells ***(37).***

CD69 in different hematological malignancies:

I. CD69 in T Cell Lymphoma.

Dorfman et al. reported immunohistochemical staining for CD69 in frozen and paraffin sections of peripheral T cell lymphomas that exhibit

immunoreactivity for markers of Th1 or Th2 differentiation. CD69 expression correlated with immunoreactivity for other Th1 differentiation markers in 18 of 19 frozen specimens of peripheral T cell lymphomas (P = 0.0005). In ten of these cases in which paraffin-embedded tissue was available for study, CD69 immunohistochemical staining of paraffin sections correlated with frozen section expression ***(38)***.

CD69 immunostaining was performed on paraffin sections from 53 additional cases of peripheral T cell lymphoma and correlated with immunoreactivity for other Th1 differentiation markers and was associated with specific subtypes of peripheral T cell lymphoma, including angioimmunoblastic lymphoma, Lennert's lymphoma, and mycosis fungoides/Sezary syndrome, previously noted to express Th1 differentiation-associated markers. Anaplastic large cell lymphoma, both systemic and cutaneous, which typically exhibits immunoreactivity for markers of Th2 expression, was negative for CD69 immunostaining in 22 of 24 cases. CD69 immunostaining results support previous findings that a subset of T cell lymphomas exhibits immunophenotypic features of either Th1 or Th2 T cell differentiation. In addition, CD69 is a useful immunohistochemical marker for specific T cell lymphomas in frozen and paraffin-embedded tissue ***(38).***

II.CD69 in B-cell non-Hodgkin's lymphoma.

The activation associated proteins CD23 and CD69 are expressed on cells of different lineages upon mitogenic stimulation. CD23 is a well characterized multifunctional protein in lymphocyte development recognized as a diagnostic marker for chronic lymphocytic leukaemia. Ninety samples from 84 patients with NHL of B cell type were studied by Erlanson, M. et al. for the expression of CD23 and CD69 in CD20+ B cells by flow cytometric dual parameter analysis. In individual lymphomas the CD23 and CD69 antigens showed an "on or off" pattern with most or very few cells positive for each antigen. The CD23 antigen was expressed in 23 of 53 (43%) indolent lymphomas and in 2 of 37 (5%) aggressive cases. Most indolent lymphomas (81%) and about half the aggressive cases (53%) expressed the CD69 antigen. Thus, both markers were associated with indolent type. CD23 expression correlated with chronic lymphocytic leukaemia subtype and CD69 expression with male gender, advanced stage, newly diagnosed lymphoma and shorter survival***(39)***

III. CD69 in Multiple Myeloma

Multiple myeloma is an incurable disease characterized by proliferation of clonal malignant plasma cells (PC). The neoplastic plasma cells (PC) develop bortezomib resistance, they have a germinal center B cell like immunophenotype, including decreased to absent expression of

CD69. The activation antigen CD69 associates with and inhibits the function of Sphingosine 1-phosphate (S1P). Other data showed that MM cells express the S1P receptors, S1P1, S1P2 and S1P3. Furthermore, S1P protects MM cells against dexametason-induced apoptosis. Importantly, S1P upregulates Mcl-1 expression in a time and concentration-dependent manner in human MM cell lines***(40).***

IV.CD69 in Acute Myeloid Leukemia (AML)

In acute myeloid leukemia (AML), leukemia stem cells (LSCs) have self-renewal potential and are responsible for relapse. In AML, CD69 expression marks an LSC-enriched subpopulation with enhanced in vivo self-renewal capacity. Cytometry by time of flight (CyTOF) was used to define activated signaling pathways in LSC subpopulations in AML. Furthermore, Antony et al compared the signaling activation states of CD69 High and CD36 High subsets of primary human AML. The human CD69 High subset expresses low levels of Ki67 and high levels of NFκB. Additionally, the human CD69 High AML subset also has enhanced colony-forming capacity. Bayesian network modeling was applied to compare the global signaling network within the human AML subsets. It is found that distinct signaling states, distinguished by NFκB level, correlate with divergent functional subsets, defined by CD69 and CD36 expression, in human AML. Targeting NFκB with proteasome inhibition diminished colony formation ***(41)***.

V.CD69 in Chronic Myeloid Leukemia (CML)

In oncological studies, CD69 levels are elevated in several hematopoietic malignancies, including CML cells**.** CD69 was detected on BCR-ABL-positive CML cell line K562 and primary CML stem cells ***(42, 43).*** Imatinib treatment decreases the protein level of CD69, suggesting that CD69 is a downstream target of BCR-ABL ***(42).***

VI.CD69 in T Cell Leukemia

Human T-cell leukemia virus type 1 (HTLV-1) infection is associated with the development of adult T-cell leukemia (ATL) and various inflammatory diseases. CD69 is a marker of early activation of lymphocytes. The *CD69* gene was upregulated in all viral protein Tax-expressing HTLV-1-transformed T-cell lines, except MT-2 and peripheral blood mononuclear cells from patients with ATL compared with uninfected T-cell line, Tax-negative ATL-derived T-cell lines and normal peripheral blood mononuclear cells. Flow cytometric analysis and immunohistochemical analysis confirmed the enhanced expression of CD69 in HTLV-1-transformed T-cell lines and in ATL cells in lymph nodes and skin lesions, and its absence in MT-2 and peripheral blood mononuclear cells ***(44)***.

CD69 expression was induced following infection of human T-cell line with HTLV-1, and specifically by Tax. Tax transcriptionally activated *CD69* gene through both nuclear factor-κB and cyclic adenosine 3′,5′-monophosphate response element-binding protein signaling pathways.

Detailed analysis of the CD69 promoter indicated that the Tax-induced expression of *CD69* was regulated by multiple cis-acting elements and by the interplay of transcription factors of the nuclear factor-κB, early growth response and cyclic adenosine 3′,5′-monophosphate response element-binding protein families. The lack of CD69 expression in MT-2 is due to epigenetic
mechanism involving deacetylation, but not methylation. So CD69 is considered as a Tax-regulated gene, and its regulation by Tax may play a role in cellular activation and HTLV-1-induced disease pathogenesis***(44).***

CD69 as a prognostic marker in Chronic lymphocytic leukemia (CLL)

The clinical course of patients with B-cell chronic lymphocytic leukemia (CLL) is quite variable and the two major clinical staging systems are unable to prospectively discriminate an indolent or aggressive course within the low and intermediate-risk categories. For this reason, several biological parameters have been added to the staging systems to differentiate prognostic subsets. Several publications reported the prognostic significance of specific immunoglobulin variable heavy chain (IGHV) gene features in CLL. However, since IGHV gene sequencing remains a demanding technique, many studies focused on the identification of alternative markers with similar prognostic value the expression of which is easier to investigate, such as CD38 and T-cell specific zeta-associated protein-70 (ZAP-70)***(45)***.

CD69 demonstrated true additive prognostic properties, since the CD69+ plus ZAP-70+ or CD38+ or immunoglobulin variable heavy chain gene unmutated patients had the worst progression free survival and overall survival. Interestingly, low CD69 expression was necessary to correctly prognosticate the longer progression free survival of patients with a low tumor burden of β2-microglobulin, of soluble CD23, or of Rai stages 0-I. CD69 was confirmed to be an independent prognostic factor in multivariate analysis of progression free survival and overall survival. Clinically, CD69 positive chronic lymphocytic leukemias received chemotherapy more frequently, and presented a shorter duration of response after fludarabine plus rituximab as well as shorter progression free survival and overall survival***(45).***

Various research showed the importance of CD69 and CD38 expression as prognostic indicators in CLL. D38 is a transmembrane glycoprotein that is normally expressed at several stages of B cell maturation, precursor B cells, follicular center cells, and plasma cells. Normally, CD38 can induce proliferation or apoptosis signaling depending on stage of cell maturation, nature of stimulus, and density of this marker expression with cofactor so that CD38 plays critical role in fate of leukemic B cells. It is found higher expression in tumor microenvironment. Its expression is variable and has received

attention as prognostic indicator. Higher expression was associated with advanced stage disease, poor response to chemotherapy with shorter overall survival***(46).***

CD95 is the best described cell surface antigen associated with apoptotic pathways, CD95 expression on B lymphocyte is analyzed before and after the in vitro induction of leukemia with phorbol-12-myristate 13-acetate (PMA), ionomycin, phytohemagglutinin, or myogen. The degree of activation was monitored on the basis of the early activation marker (CD69) expression. Chronic lymphocytic leukemia cells were revealed to be characterized by a markedly lower expression of CD95 than normal lymphocytes. Additionally, the leukemia cells were incapable of enhancing the expression of this molecule upon activation; although, it must be mentioned that such phenomenon was documented in the case of normal lymphocytes. Both CLL cells and normal lymphocytes showed a higher
expression of CD69 after stimulation. Consequently, these findings suggest that CLL cells express the early activation marker to a similar extent as normal cells, but their activation-induced apoptosis is impaired ***(47).***

CD69 expression potentially predicts response to bendamustine. Clinical responses to bendamustine in chronic lymphocytic leukemia (CLL) are highly heterogeneous and no specific markers to predict sensitivity to this drug have been reported. In order to identify biomarkers of response, the *in vitro* activity of bendamustine and the gene expression profile in primary CLL cells was analyzed. It is observed that mRNA expression of CD69 and *ITGAM* (CD11b) constitute the most powerful predictor of response to bendamustine. When we interrogated the predictive value of the corresponding cell surface proteins, the expression of the activation marker CD69 was the most reliable predictor of sensitivity to bendamustine. Importantly, a multivariate analysis revealed that the predictive value of CD69 expression was independent from other clinico-biological CLL features. It is observed that when CLL cells were co-cultured with distinct subtypes of stromal cells, an upregulation of CD69 was accompanied by a reduced sensitivity to bendamustine ***(48)***.

In agreement with this, tumor cells derived from lymphoid tumor niches harbored higher CD69 expression and were less sensitive to bendamustine than their peripheral blood counterparts. Furthermore, pretreatment of CD69 high CLL cases with the B-cell receptor (BCR) pathway inhibitors ibrutinib and idelalisib decreased CD69 levels and enhanced bendamustine cytotoxic effect. CD69 could be a predictor of bendamustine response in CLL patients, and the combination of clinically tested BCR signaling inhibitors with bendamustine may represent a promising strategy for bendamustine low responsive CLL cases ***(48)***.

References:

1. Ziegler, S. F., Ramsdell, F., Hjerrild, K. A. et al. (1993). Molecular characterization of the early activation antigen CD69: a type II membrane glycoprotein related to a family of natural killer cell activation antigens. Eur. J. Immunol. 23:1643.
2. Kimura, M. Y., Hayashizaki, K., Tokoyoda, K., Takamura, S., Motohashi, S. and Nakayama, T. (2017). Crucial role for CD69 in allergic inflammatory responses: CD69-Myl9 system in the pathogenesis of airway inflammation. Immunol. Rev. 278:87.
3. Mackay, L. K. and Kallies, A. (2017). Transcriptional regulation of tissue-resident lymphocytes. Trends Immunol. 38:94.
4. Fan, X. and Rudensky, A. Y. (2016). Hallmarks of tissue-resident lymphocytes. Cell 164:1198.
5. Hayashizaki, K., Kimura, M. Y., Tokoyoda, K. et al. (2016). Myosin light chains 9 and 12 are functional ligands for CD69 that regulate airway inflammation. Sci. Immunol. 1:eaaf9154.
6. Cyster, J. G. and Schwab, S. R. (2012). Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. Annu. Rev. Immunol. 30:69.
7. Shiow, L. R., Rosen, D. B., Brdickova, N., Xu, Y., An, J., Lanier, L. L., Cyster, J. G. et al. (2006), CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. Nature. 440: 540– 544.
8. Bankovich, A. J., Shiow, L. R. and Cyster, J. G. (2010), CD69 suppresses sphingosine 1-phosophate receptor-1 (S1P1) function through interaction with membrane helix 4. J. Biol. Chem. 285: 22328–22337.
9. Wei, S. M., Pan, H. L., Wang, L. et al. (2017). Combination therapy with dendritic cell-based vaccine and anti-CD69 antibody enhances antitumor efficacy in renal cell carcinoma-bearing mice. Turk. J. Med. Sci. 47:658.
10. Esplugues, E., Vega-Ramos, J., Cartoixà, D. et al. (2005). Induction of tumor NK-cell immunity by anti-CD69 antibody therapy. Blood 105:4399.
11. Pauken, K. E. and Wherry, E. J.(2015). Overcoming T cell exhaustion in infection and cancer. Trends Immunol. 36:265.
12. Gonzalez-Amaro, R., Cortes, J. R., Sanchez-Madrid, F. and Martin, P.,(2013) Is CD69 an effective brake to control inflammatory diseases? Trends Mol. Med. 2013. 19: 625–632.
13. Radstake, T. R., van Bon, L., Broen, J., Hussiani, A., Hesselstrand, R., Wuttge, D. M., Deng, Y. et al.(2009), The pronounced Th17 profile in systemic sclerosis (SSc) together with intracellular expression of TGFbeta and IFNgamma distinguishes SSc phenotypes. PLoS One 2009. 4: e5903.
14. Alari-Pahissa, E., Notario, L., Lorente, E., Vega-Ramos, J., Justel, A., Lopez, D., Villadangos, J. A. et al.(2012), CD69 does not affect the extent of T cell priming. PLoS One 2012. 7: e48593.

15. Radulovic, K., Manta, C., Rossini, V., Holzmann, K., Kestler, H. A., Wegenka, U. M., Nakayama, T. et al. (2012), CD69 regulates type I IFN-induced tolerogenic signals to mucosal CD4 T cells that attenuate their colitogenic potential. J. Immunol. 2012. 188: 2001–2013.
16. Hasegawa, A., Iwamura, C., Kitajima, M., Hashimoto, K., Otsuyama, K., Ogino, H., Nakayama, T. et al. (2013), Crucial role for CD69 in the pathogenesis of dextran sulphate sodium-induced colitis. PLoS One 2013. 8: e65494.
17. Feng, C., Woodside, K. J., Vance, B. A., El-Khoury, D., Canelles, M., Lee, J., Gress, R. et al. (2002), A potential role for CD69 in thymocyte emigration. Int. Immunol. 14: 535–544.
18. Nakayama, T., Kasprowicz, D. J., Yamashita, M., Schubert, L. A., Gillard, G., Kimura, M., Didierlaurent, A. et al. (2002), The generation of mature, singlepositive thymocytes in vivo is dysregulated by CD69 blockade or overexpression. J. Immunol. 168: 87–94.
19. Lauzurica, P., Sancho, D., Torres, M., Albella, B., Marazuela, M., Merino, T., Bueren, J. A. et al. (2000), Phenotypic and functional characteristics of hematopoietic cell lineages in CD69-deficient mice. Blood 2000. 95: 2312–2320.
20. Laffo´n, A. et al. (1991) Upregulated expression and function of VLA-4 fibronectin receptors on human activated T cells in rheumatoid arthritis. J. Clin. Invest. 88, 546 552
21. Remmers, E.F. et al. (1996) A genome scan localizes five non-MHC loci controlling collagen-induced arthritis in rats. Nat. Genet. 14, 82–85
22. Mc Indoe, R.A. et al. (1999) Localization of non-MHC collagen-induced arthritis susceptibility loci in DBA/1j mice. Proc. Natl. Acad. Sci. U. S. A. 96, 2210–2214
23. Sancho, D., Gomez, M., Viedma, F., Esplugues, E., Gordon-Alonso, M., Garcia-Lopez, M. A., de la Fuente, H. et al. (2003), CD69 downregulates autoimmune reactivity through active transforming growth factor-beta production in collagen-induced arthritis. J. Clin. Invest. 2003. 112: 872–882.
24. Esplugues, E., Sancho, D., Vega-Ramos, J. et al. (2003). Enhanced antitumor immunity in mice deficient in CD69. J. Exp. Med. 197:1093.
25. Grewal, J.S. et al. (1999) Serotonin 5-HT2A receptor induces TGF-b1 expression in mesangial cells via ERK: proliferative and fibrotic signals. Am. J. Physiol. Renal Physiol. 276, F922–F930
26. Zingoni, A. et al. (2000) CD69-triggered ERK activation and functions are negatively regulated by CD94/NKG2-A inhibitory receptor. Eur. J. Immunol. 30, 644–651.
27. Gorelik, L. and Flavell, R.A. (2001) Immune-mediated eradication of tumors through the blockade of transforming growth factor-b signaling in T cells. Nat. Med. 7, 1118–1122
28. de la Fuente, H., Cruz-Adalia, A., Martinez Del Hoyo, G., Cibrian-Vera, D., Bonay, P., Perez-Hernandez, D.,

Vazquez, J. et al. (2014), The leukocyte activation receptor CD69 controls T cell differentiation through its interaction with galectin-1. Mol. Cell Biol. 34: 2479–2487.

29. Toscano, M. A., Bianco, G. A., Ilarregui, J. M., Croci, D. O., Correale, J., Hernandez, J. D., Zwirner, N. W. et al.(2007), Differential glycosylation of TH1,TH2 and TH-17 effector cells selectively regulates susceptibility to cell death. Nat. Immunol. 2007. 8: 825–834.
30. Cedeno-Laurent, F., Opperman, M., Barthel, S. R., Kuchroo, V. K. and Dimitroff, C. J.(2012), Galectin-1 triggers an immunoregulatory signature in Th cells functionally defined by IL-10 expression. J. Immunol. 188:c3127–3137.
31. Lin, C. R., Wei, T. Y., Tsai, H. Y., Wu, Y. T., Wu, P. Y., & Chen, S. T. (2015). Glycosylation-dependent interaction between CD69 and S100A8/ S100A9 complex is required for regulatory T-cell differentiation. The FASEB Journal, 29, 5006–5017.
32. Cibrian, D., Saiz, M. L., de la Fuente, H., Sanchez-Diaz, R., MorenoGonzalo, O., Jorge, I., Ferrarini, A. et al. (2016), CD69 controls the uptake of L-tryptophan through LAT1-CD98 and AhR-dependent secretion of IL-22 in psoriasis. Nat. Immunol. 2016. 17: 985–996.
33. Spiegel, S. and Milstien, S. (2011), The outs and the ins of sphingosine-1-phosphate in immunity. Nat. Rev. Immunol. 11: 403–415.
34. Hunter, M. C., Teijeira, A. and Halin, C. (2016), T cell trafficking through Shinoda, K., Tokoyoda, K., Hanazawa, A., Hayashizaki, K., Zehentmeier, S., Hosokawa, H., Iwamura, C. et al. (2012), Type II membrane protein CD69 regulates the formation of resting T-helper memory. Proc. Natl. Acad. Sci. U. S. A. 2012. 109: 7409–7414.
35. Hervier, B., Beziat, V., Haroche, J., Mathian, A., Lebon, P., Ghillani-Dalbin, P., Musset, L. et al. (2011), Phenotype and function of natural killer cells in systemic lupus erythematosus: excess interferon-gamma production in patients with active disease. Arthritis. Rheum. 63: 1698–1706.
36. Shinoda, K., Tokoyoda, K., Hanazawa, A., Hayashizaki, K., Zehentmeier, S., Hosokawa, H., Iwamura, C. et al. (2012), Type II membrane protein CD69 regulates the formation of resting T-helper memory. Proc. Natl. Acad. Sci. U. S. A. 2012. 109: 7409–7414.
37. Lamana, A., Martin, P., de la Fuente, H., Martinez-Munoz, L., CruzAdalia, A. (2011), Ramirez-Huesca, M., Escribano, C. et al., CD69 modulates sphingosine-1-phosphate-induced migration of skin dendritic cells. J. Invest. Dermatol. 131: 1503 1512.
38. Dorfman. D. M., Shahsafaei. A., - Hum Pathol – (2002) CD69 expression correlates with expression of other markers of Th1 T cell differentiation in peripheral T cell lymphoma. 33 (3); 330-4.
39. Erlanson M, Grönlund E, Löfvenberg E, Roos G, Lindh J - Eur J Haematol – (1998). Expression of activation markers

CD23 and CD69 in B-cell non-Hodgkin's 60 (2); 125-32.

40. Gabriele Buda, Giovanni Carulli, Enrico Orciuolo, Paola Sammuri, Sara Galimberti, Martina Rousseau, Francesca Martini, Mario Petrini (2015), CD69 Expression Predicts Favorable Outcome in Multiple Myeloma Patients Treated with VTD, Blood, Volume 126, Issue 23, Page 1768.
41. Antony, M. L. Chang, D. Noble-Orcutt, K. E. Kay, A. Jensen, J. L. Mohei, H. Myers, C. L. Sachs, K. Sachs, Z. Journal; Leukemia and Lymphoma, (2023). CD69 marks a subpopulation of acute myeloid leukemia with enhanced colony forming capacity and a unique signaling activation state, DOI 10.1080/10428194.2023.2207698.
42. Hantschel, O., Gstoettenbauer, A., Colinge, J., Kaupe, I., Bilban, M., Burkard,
T. R., et al (2008). The chemokine interleukin-8 and the surface activation protein CD69 are markers for Bcr-Abl activity in chronic myeloid leukemia. Molecular Oncology, 2, 272–281.
43. Wisniewski, D., Affer, M., Willshire, J., & Clarkson, B. (2011). Further phenotypic characterization of the primitive lineage- CD34+CD38-CD90+CD45RA- hematopoietic stem cell/progenitor cell sub-population isolated from cord blood, mobilized peripheral blood and patients chronic myelogenous leukemia. Blood Cancer Journal, 1, e36.
44. Chie Ishikawa, Hirochika Kawakami, Jun-Nosuke Uchihara, Masachika Senba, Naoki Mori (2013). CD69 overexpression by human T-cell leukemia virus type 1 Tax transactivation, Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, Volume 1833, Issue 6, Pages 1542-1552.
45. Del Poeta, G., et al. (2012). "CD69 is independently prognostic in chronic lymphocytic leukemia: a comprehensive clinical and biological profiling study." Haematologica 97(2): 279-287.
46. Aref, S., et al. (2019). "Prognostic relevance of CD69 expression in B cell chronic lymphocytic leukemia." Comparative Clinical Pathology 28: 33-40.
47. Grywalska, E., et al. (2018). "Relationship between the expression of CD25 and CD69 on the surface of lymphocytes T and B from peripheral blood and bone marrow of patients with chronic lymphocytic leukemia and established prognostic factors of this disease." Adv Clin Exp Med 27(7): 987-999.
48. Montraveta, A., et al. (2016). "CD69 expression potentially predicts response to bendamustine and its modulation by ibrutinib or idelalisib enhances cytotoxic effect in chronic lymphocytic leukemia." Oncotarget 7(5): 5507-5520.