# CD49d in chronic lymphocytic leukemia: a molecule with multiple regulation layers. Comment to "Sialylation regulates migration in chronic lymphocytic leukemia"

We read with interest the study by Natoni *et al*. on the regulation of migration in chronic lymphocytic leukemia (CLL) cells by sialylation, recently published in Haematologica.[1]

In the study, the authors investigate the sialylation status of CLL cells, evaluating specific expression of α2-3 and α2-6 sialic acids, demonstrating that this particular modification is highly represented on CLL cells.[1] Furthermore, they show that sialylation can affect the migratory capacity of CLL cells toward VCAM-1 and fibronectin (FN), which, in turn, is effectively suppressed upon treatment with neuraminidase (a glycoside hydrolase specific for sialic acids). Notably, VCAM-1 and FN are two known cognate ligands for the CD49d integrin, which is identified in this work as highly sialylated.

Sialylation, the addition of negatively-charged sialic acids onto N-linked and O-linked protein glycans is a frequent post-translational modification for cell membrane proteins involved in cell-cell and cell-matrix interactions. It occurs within the Golgi network during protein maturation and is regulated by a wide variety of substrate-specific sialyltransferases, which are frequently found dysregulated in cancer.[2]

In the B-cell context, known sugar modifications include mannosylation of the B-cell receptor (BCR)-influencing antigen engagement and signaling,[3] and, as reported by Natoni's group, sialylation of the CD49d integrin in multiple myeloma[4] and in CLL.[1]

The latter observation is particularly relevant, since CD49d represents, along with mutations of IGHV genes, *TP53* and *NOTCH1*, one of the major biological prognosticators in CLL, and patients affected by a CD49d-expressing CLL (about 40% of all cases) usually experience a worse clinical outcome.[5]

Functionally, CD49d is the α4 chain of the α4/β1 complex (CD49d/CD29) composing the very late antigen-4 (VLA-4) integrin. VLA-4 operates as a cell-cell and cell-matrix receptor by interacting with its specific ligands: VCAM-1 involved in cell-cell interactions, and FN and EMILIN1 both involved in cell-matrix interactions. All three ligands are present in CLL-involved tissues and are available for VLA-4 engagement.[6]

As one of the major actors regulating CLL microenvironmental interactions, CD49d expression and function are regulated at multiple levels, both genetic and epigenetic, as well as through protein-protein cooperation and/or conformational changes. In this complex scenario, sialylation, as described by Natoni and colleagues,[1] may represent the last regulation identified of the many layers of biochemical modifications and/or physical interactions of CD49d with other molecules, which can eventually contribute to fine-tune CD49d-mediated activities such as migration and adhesion.

Along this line, particularly illustrative is the case of trisomy 12 CLL. This subset is characterized by peculiar features of microenvironmental dependency, lymph node involvement and frequent Richter transformation, which includes *NOTCH1* mutations, expression of (BCR) with stereotyped features (subset 8) and elevated expression of several integrins.[7] Among these, CD49d stands out as almost universally expressed in this trisomy 12 CLL, mainly due to a methylation-dependent epigenetic regulation mechanism of the *ITGA4* gene (encoding for CD49d).[7] As another layer of expression control, upregulation of CD49d expression in CLL also occurs outside the trisomy 12 subset, involving NF-κB pathway activation via NOTCH1 mutations. In fact, *NOTCH1* mutation or *in vitro* activation of the *NOTCH1* pathway leads to nuclear translocation of the NF-κB RelA subunit and upregulation of CD49d levels, whereas inhibition of the NF-κB pathway results in a downregulation of the integrin expression.[8]

A functional layer of regulation of the activity of the expressed CD49d molecule may come from its interaction with CD38, another microenvironmental receptor known to be expressed by CLL cells especially in cases with a bad prognosis.[9] CD38, often found simultaneously expressed with CD49d, has a propensity to form with CD49d supramolecular complexes on the cell surface that potentiate CD49d-mediated adhesion and spreading.[10] CD49d and CD38 are also interconnected by a sort of "humoral" link, as CD38 engagement by its counter receptor CD31 results in the production by CLL cells of two chemokines (CCL3 and CCL4) which in turn recruit monocyte/macrophages that, by secreting TNFα, contribute to upregulate the stromal/endothelial expression of VCAM-1, thus favoring CD49d/VCAM-1 adhesion and CLL cell survival.[11]

Another known layer regulating CD49d activities is represented by the activation of VLA-4 via "inside-out" through BCR stimulation, a functional mechanism present in CLL cells as a remnant of an identical mechanism occurring in secondary lymphoid organs during the antigen-dependent maturation process of normal B lymphocytes.[12] In this context, stimuli originating from a fit BCR/antigen interaction can activate VLA-4 to promote cell adhesion to VCAM-1-expressing follicular dendritic cells, such a tonic interaction providing rescue of B cells from apoptosis and promote their differentiation.[12]

The interaction between BCR and VLA-4 in CLL could be relevant, given the increasing use of BCR signaling inhibitors

such as ibrutinib or acalabrutinib for most CLL patients.[6] These drugs have the common feature of favoring lymph node shrinkage and the release of lymphocytes in the blood stream, allegedly by hampering integrin-dependent adhesion mechanisms. However, CD49d-expressing CLL show reduced redistribution lymphocytosis and nodal shrinkage, a clinical manifestation of the residual capability of VLA-4 integrin to be activated at high affinity via BCR-mediated inside-out mechanism, thus promoting CLL cell retention.[6] This also becomes evident by the higher expression of CD49d within the bone marrow and proliferative compartments, as defined by the relative expression of CD5 and CXCR4 markers.[13]

These (epi)genetic- and protein-regulated mechanisms, tightly intertwine with the recently described sialylation of CD49d in CLL cells, as reported by Natoni *et al.*[1] which provides a novel, post-translational layer of regulation of integrin dynamics in CLL.

Integrin sialylation has been described in a variety of tumors, and associated with increased migration potential, metastasis, immune evasion and cell survival. As an example, sialylation of the VLA-5 integrin ($\alpha5/\beta1$) preserved colon adenocarcinoma cells from anoikis (apoptosis in response to lack of external stimuli).[2] Although most of the research on sialylated integrins has been conducted within solid tumors, the studies by Natoni *et al*. point also at a significant role for this modification in hematological malignancies, with the reports of CD49d sialylation in CLL and in multiple myeloma.[1,4] Much research is still needed to fully understand the functional dynamics of integrins in CLL, where VLA-4 expression represents one of the major actors of microenvironmental interactions, thus mediating proliferation, survival and drug resistance.

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**Disclosures**

No conflicts of interest to disclose.

**Contributions**

FP and VG wrote and revised the manuscript. ET and AZ contributed to writing and revising the manuscript.

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