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Editorial

Editorial: **Targeting JAM-C on mantle cell lymphoma B cells: time for clinical testing?**

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MCL is a rare non-Hodgkin B cell lymphoma affecting patients in their 60s, with a male-to-female ratio of 4:1. The cell of origin of MCL is usually considered a naïve, pre-GC B cell, homing in primary lymphoid follicles or in the mantle region of secondary lymphoid follicles. Two different pathways of MCL development have been identified: namely, 1) classic MCL, the most frequent variant, that carries unmutated or minimally mutated variable regions of IGHVs, expresses the transcription factor SOX11 and involves lymph nodes, as well as other extranodal sites, and 2) MCL, characterized by mutated IGHV and high SOX11 expression, which manifests as leukemic, non-nodal MCL, infiltrating the peripheral blood, bone marrow, and spleen. This latter MCL variant, which affects a minority of patients, presumably undergoes IGHV somatic hypermutation in the GC. MCL is an indolent disease that can become aggressive as a result of the occurrence of secondary genetic abnormalities, often involving p53. Higher than 90% of MCL cases shows the typical t(11;14)(q13:q32) chromosomal translocation, whereby Cyclin D1 is overexpressed, leading to accumulation of highly proliferating cells [1].

The key role played by the tumor microenvironment in promoting cancer cell growth and chemoresistance is now well established. MCL cells adhere to MSCs in a VLA-4 (CD49d)-dependent fashion, and this interaction protects malignant B lymphocytes from the toxic effects of chemotherapy. IL-6 secreted by MCL cells and MSC contributes to chemoresistance by activating the JAK/STAT3 and PI3K/Akt pathways that promote MCL growth and survival [2]. In addition, MCL cells suppress anti-tumor immune responses by up-regulating PD-L1, the ligand for the PD-1 immune checkpoint, and recruiting $CD4^+$, $CD25^+$, forkhead box $p3^+$ T regulatory cells (**Fig. 1**). In this respect, it has recently been shown that MCL cells express a "signature" represented by the overexpression of the following: 1) CCL4, implicated in the recruitment of T regulatory cells, 2) CCL5, a T cell chemoattractant, and 3) 4-1BB-L, the ligand of the costimulatory molecule 4-1BB involved in B cell activation [3].

To home to the tumor microenvironment, malignant cells must express adhesion molecules and chemokine receptors. The former molecules are essential to allow binding of circulating tumor cells to the endothelium, whereas chemokine receptors are instrumental for cell attraction along chemokine gradients. During recruitment, normal and malignant leukocytes migrate out of the circulation through a multistep process known as TEM, mediated by adhesive events between inflammatory cytokine-activated endothelium and leukocyte surface proteins. Numerous families of molecules, such as selectins, integrins, and cell adhesion molecules participate in TEM by establishing mostly heterophilic interactions.

We [4] and others [5] have shown that MCL cells express the chemokine receptors CCR1–CCR7 and CXCR1–CXCR5, with patterns similar to those detected in normal circulating $CD5^+$ B cells, the presumed normal counterparts of most MCL cases. However, in our study, 2 chemokine receptors only were found to promote in vitro chemotaxis of MCL cells consistently, i.e., CCR7, which binds CCL19 and CCL21, and CXCR4, whose major ligand is CXCL12 [4]. Notably, CCR7 is essential for migration of normal and malignant B cells to lymph nodes, and CXCL12 is involved together with VLA-4 in the adhesion of MCL cells to MSC [5]. Other investigators have shown that CXCR5 is also functional in MCL cells [5].

JAMs are glycoproteins belonging to the Ig superfamily comprised of 2 extracellular Ig-like domains, 1 transmembrane domain, and a cytoplasmic tail of variable length. JAM-A, JAM-B, and JAM-C display up to 35% identity of the amino acid sequence and share a short intracellular domain of 40–50 residues containing a class II pancreatic-duodenal homeobox factor 1-binding motif at the C terminus. JAMs are generally expressed in subsets of leukocytes (e.g., activated T cells and NK cells) and epithelial and

Abbreviations: GC = germinal center, IGHV = Ig heavy chain variable region, JAM = junctional adhesion molecule, JAM-$C^{-/-}$ = junctional adhesion molecule deficient, MCL = mantle cell lymphoma, MSC = mesenchymal stromal cell, PD1 = programmed cell death protein 1, TEM = transendothelial migration

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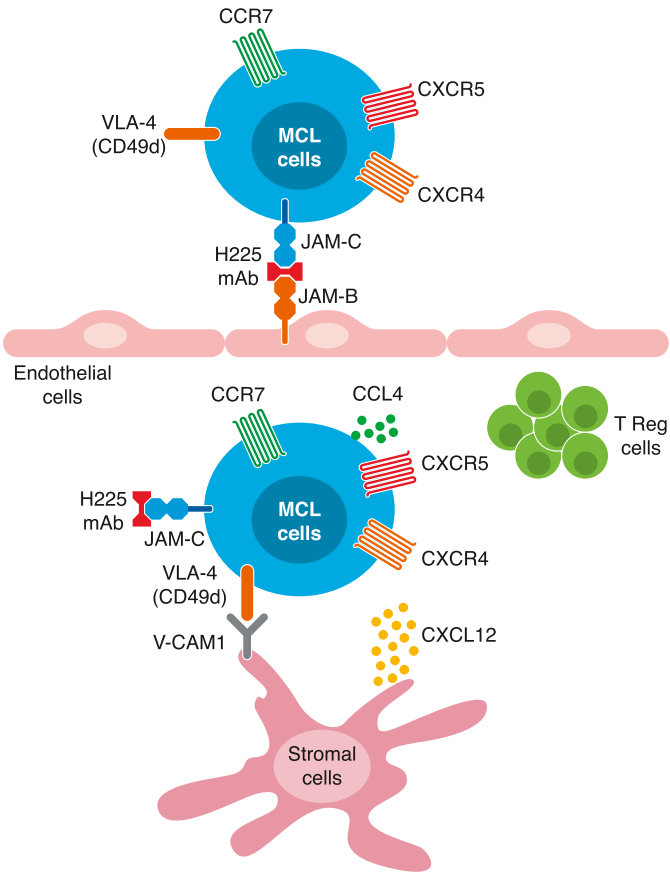

Figure 1. A schematic view of the functional consequences of JAM-C targeting with H225 mAb. (Upper) An MCL cell in the process of transmigrating through an endothelial vessel to access a secondary lymphoid organ, where tumor microenvironment is depicted (lower). The JAM-C-specific H225 mAb blocks the interaction between JAM-C on the MCL cell and JAM-B on the endothelial cell, thus preventing transmigration of tumor cells. Furthermore, the H225 mAb inhibits proliferation of MCL cells in the tumor microenvironment, where these cells have been attracted by chemokines binding to CXCR4, CCR7, and CXCR5. In particular, the figure shows a gradient of the CXCR4 ligand CXCL12 produced by MSCs. The latter cells interact with MCL cells through binding of VCAM1 to VLA-4. Regulatory T cells (T_{regs}), which represent another important component of the MCL microenvironment, are here attracted by tumor cell-derived CCL4.

endothelial cells. As for the latter cells, JAM-C has been localized at tight junctions and found to be expressed by high endothelial venules and lymphatic vessels in lymphoid organs. JAMs contain a dimerization motif that allows formation of *cis*-homodimers of JAM-B and JAM-C in the course of endothelial intercellular contacts. On the other hand, JAM-B establishes heterophilic interactions with JAM-C and the VLA-4 integrin, and endothelial JAM-C can bind the membrane-activated complex 1 (CD11b/CD18) integrin on leukocytes [6].

It is well established that JAMs are involved in the control of vascular permeability, leukocyte TEM, angiogenesis, and tumor progression [6]. In this respect, it has been shown that JAM-C is expressed by human melanoma cells, both primary and metastatic to the lung. In the B16 melanoma mouse model, JAM-C, expressed by melanoma cells and endothelial cells, promoted TEM of tumor cells. JAM-$C^{-/-}$ mice, as well as endothelial cell-specific JAM-$C^{-/-}$ mice, showed decreased metastasis of B16 melanoma to the lung [7].

Matthes and coworkers [8] have conducted a series of studies on the expression and function of JAM-C in normal and malignant B cells. They have shown that JAM-C is expressed by normal B cells and that such expression allows distinction of $CD27^+$ memory B cells homing in the GC from those located outside of the GC, the former JAM-C^- and the latter JAM-C^+. Furthermore, 2 categories of B cell malignancies were identified on the ground of JAM-C expression, i.e., JAM-C^- tumors, including chronic lymphocytic leukemia, follicular lymphoma, and diffuse large B cell lymphoma, and JAM-C^+ tumors, including marginal zone B cell lymphoma, MCL, and hairy cell leukemia [8]. As JAM-C plays a role in neutrophil and monocyte migration, Matthes and coworkers [8] investigated whether JAM-C was also involved in B cell migration. With the use of an in vivo assay, they showed that affinity-purified rabbit anti-JAM-C antibodies decreased the homing of B cells to bone marrow, spleen, and lymph nodes. This effect was complementary to that of the anti-VLA-4 antibody, which decreased B cell homing to bone marrow and lymph node but not to the spleen. When JAM-C^+ lymphoma B cells were tested in the same assay, the results obtained were similar to those achieved with normal B cells, but the inhibition of lymph node homing was of a higher degree. Finally, anti-JAM-C antibodies blocked the adhesion of JAM-C^+ B cells to JAM-B vascular endothelial cells, indicating that JAM-C/JAM-B heterophilic interactions rather than JAM-C/JAM-C homophilic interactions were involved in this experimental system [9].

In this issue of the *Journal of Leukocyte Biology*, Matthes and coworkers [10] have investigated in depth the effects of JAM-C on the in vivo growth and dissemination of MCL cells and developed a novel, preclinical therapeutic strategy based on newly produced anti-JAM-C mAb. Two of these mAb—H225 and H36.6—efficiently inhibited the homing of normal B cells to secondary lymphoid organs, but the activity of H225 mAb proved to be the strongest. MCL tumors were generated in SCID/NOD mice by intravenous injection of the Jeko-1 cell line. Treatment of mice with H225 mAb, administered 6 d after tumor cell inoculum, virtually abolished MCL cell homing to the spleen, liver, bone marrow, and lymph nodes. When H225 mAb was administered on d 10 after injection of Jeko-1 cells, the effects on spleen and liver homing persisted, whereas those on bone marrow and lymph node homing waned. Inhibition of MCL tissue homing was paralleled by reduction of tumor size. The authors hypothesized that the anti-JAM-C mAb

could affect not only homing of tumor cells but also additional intrinsic functions of the latter cells. Indeed, they demonstrated that immobilized H225 mAb inhibited in vitro the proliferation of Jeko-1 cells, as well as of primary MCL cells. Furthermore, lymphoma cells from H225-treated mice showed a clearly decreased cell proliferation compared with controls. The mechanism whereby the H225 mAb perturbed MCL cell proliferation was found to depend on reduced phosphorylation of ERK1/2.

As discussed by the authors, there are other examples of anti-tumor activity operated by inhibitors of cell migration. These include the following: 1) A6, a CD44-binding peptide, which has been successfully administered in phase 1 and 2 studies to patients with gynecologic cancers, 2) the CXCR4 inhibitor plerixafor, which is being tested in combination with conventional therapies in patients with acute lymphoblastic leukemia or chronic lymphocytic leukemia, and 3) BCR inhibitors, such as ibrutinib, that besides inhibiting BCR-mediated signaling, also dampen B cell migration. It is of note that in a xenograft model of human MCL, an anti-CCR7 mAb exerted a potent anti-tumor activity, abrogating infiltration of tumor cells in lymphoid and nonlymphoid organs [11]. This finding is consistent with our results showing that CCR7 is a "master" chemokine receptor for primary MCL cells. Finally, the anti-VLA-4 natalizumab is currently used for treatment of multiple sclerosis patients, and the above-mentioned ability of this mAb to target in vitro MCL–MSC interactions supportive of tumor cell survival may provide a rationale for a clinical trial in MCL patients [5].

The availability of novel drugs targeting different pathways, such as bortezomib, lenalidomide, and ibrutinib, will soon improve the outcome of MCL patients, but the need for further therapeutic approaches still persists [1]. Inhibition of MCL cell homing to lymphoid tissue by blocking the JAM-C molecule with mAb, such as H225, is an interesting perspective, with a sound experimental basis. It is now time to decide whether such an approach deserves to be tested in the clinical setting.

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