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Multiple Metamorphoses of CD38 from Prognostic Marker to Disease Modifier to Therapeutic Target in Chronic Lymphocytic Leukemia

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Abstract: Human CD38, an ecto-enzyme and a receptor, performs as an independent negative prognostic marker for patients with chronic lymphocytic leukemia (CLL), a hematological malignancy characterized by the accumulation of a population of mature B lymphocytes expressing CD5. Patients with a $CD38^+$ CLL clone display a more aggressive form of the disease with earlier treatment requirements and ultimately shorter overall survival than patients with a $CD38^-$ clone.

Several lines of evidence indicate that CD38 is not only a diagnostic marker but also a key element in the molecular network regulating disease maintenance and progression. First, CD38 is a receptor that induces proliferation and increases survival of CLL cells. Second, CD38 signals facilitate access of CLL cells to growth-favorable districts. This is achieved by enhancing i) chemotaxis towards CXCL12, ii) integrin-mediated adhesion and iii) matrix metalloprotease synthesis and secretion. Third, blocking monoclonal antibodies targeting CD38 impair CLL homing to spleen and bone marrow in xenograft models. These functions appear to be modulated by frontal interactions with CD31 as well as by lateral associations on the CLL membrane to form a large supramolecular complex similar to the invadosomes of epithelial cells.

Our understanding has evolved from considering CD38 as a marker of unfavorable prognosis to recognizing its function as a disease modifier. Studies in the next few years will likely determine whether the molecule can also serve as a target for new therapies, using monoclonal antibodies, inhibitors of the enzymatic activity or both.

Keywords: Chronic lymphocytic leukemia, CD38, ecto-enzymes, microenvironment, homing, therapeutic target.

FOCUS OF THE REVIEW

CD38 was recognized early on as a marker for hematological malignancies, including most cases of myeloma [1], many cases of AIDS-associated lymphoma [2], and many cases of post-transplant lymphoproliferations [3]. The majority of acute myeloid leukemia cases also express variable levels of the molecule. In the late nineties, CD38 was proposed for use as a prognostic marker for chronic lymphocytic leukemia (CLL) patients. As a basic science lab, we have adopted CLL as a disease model to gain insights into the still elusive role of the molecule in the human immune system. Functional information obtained in over a decade of investigations builds on the notion that CD38 actively modifies the nature of the disease by integrating proliferative and migratory programs. The final part of the triptych to be presented here concerns the coming of age of drugs that target CD38, currently studied in clinical trials as therapeutic agents for CLL and myeloma.

After describing the physiological role of CD38, this review discusses the main findings supporting the notion that CD38 is at the same time a clinical marker, a disease modifier and a therapeutic target for CLL patients.

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BRIEF HISTORY OF CD38

Originally defined in the early eighties as a T cell activation marker, CD38 is a surface molecule of ≈45 kDa [4]. It is widely distributed in cells of the immune system, with a see-saw pattern of expression: early hematopoietic progenitors as well as terminally differentiated cells tend to be highly positive, while resting differentiated cells are mostly negative. Expression is rapidly up-regulated following activation of mature T and B lymphocytes obtained with antigenic or polyclonal signals [reviewed in [5]].

From a historical point of view, studies on the function of the molecule diverged early on, taking two different paths. Immunologists studied CD38 using agonistic and blocking antibodies to engage the molecule and trigger measurable cellular responses. This approach proved fruitful as it revealed that upon ligation of CD38, T and B lymphocytes acquired an activated phenotype and – in some instances – initiated proliferative programs [6]. Early events included mobilization of Ca^{2+} ions, as well as phosphorylation of a number of intracellular kinases, specific for cell lineage and differentiation step. Common elements of the CD38 pathway are members of the MAP kinase family and the nuclear factor NF-kB [7-10] (Fig. **1**). On the cell surface, CD38 is a sticky protein that complexes in dimers and tetramers [11, 12] and liaises with other molecules of the cell surface. The resulting supramolecular complexes are preferentially hosted in cholesterol-rich areas of the plasma membrane [13]. Asso-

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ciated molecules in lymphocytes include T [14] and B cell receptors [13], as well as co-receptors, chemokine receptors and adhesion molecules [15-17]. Beside lateral interactions on the same cell membrane, CD38 also mediates frontal interactions with opposing cells (Fig. **1**). This was concluded after using blocking anti-CD38 antibodies to interfere with lymphocyte adhesion over an endothelial layer under dynamic conditions [18]. The result was later explained by the finding that CD38 expressed by lymphocytes interacts with CD31, a molecule abundantly expressed by endothelial cells [19, 20]. This selectin-type interaction sets in motion the adhesion cascade by activating genetic programs of lymphocytes that increase integrin expression, preparing lymphocytes for strong adhesion and extravasation.

Summing up the results of over twenty years of investigations from the immunologist's perspective two main themes emerge: the first is linked to activation and proliferation and the second concerns lymphocyte adhesion and movement.

The other path taken in CD38 studies was pursued mainly by enzymologists and was initiated by an early report describing a striking similarity in amino-acid sequence between CD38 and the enzyme ADP ribosyl cyclase (ADPRC) obtained from the sea mollusk *Aplysia Californica* [21, 22], which regulates production of cyclic ADP ribose (cADPR) from NAD. Subsequent investigations indicated that both CD38 and the *Aplysia* cyclase are novel multifunctional enzymes capable of i) cyclizing NAD to produce cADPR, ii) hydrolyzing NAD to produce ADPR and iii) synthesizing NAADP from NADP by catalyzing the exchange of nicotinamide in NADP with nicotinic acid [23, 24]. The crystal structures of both proteins have been solved [25, 26] and the critical amino acid residues shaping the active sites of the enzyme identified by site-directed mutagenesis [27, 28]. Interest in the enzymatic activity derives from the notion that the products of the reactions are potent mobilizers of Ca^{2+} from intracellular stores (cADPR and NAADP) or from the extracellular environment (ADPR). According to current model, the paradox of an extracellular enzyme synthesizing products that need to be used inside the cell could be solved by hypothesizing that cADPR, ADPR and NAADP may gain access to the cytosol through membrane pores or channels (Fig. **1**).

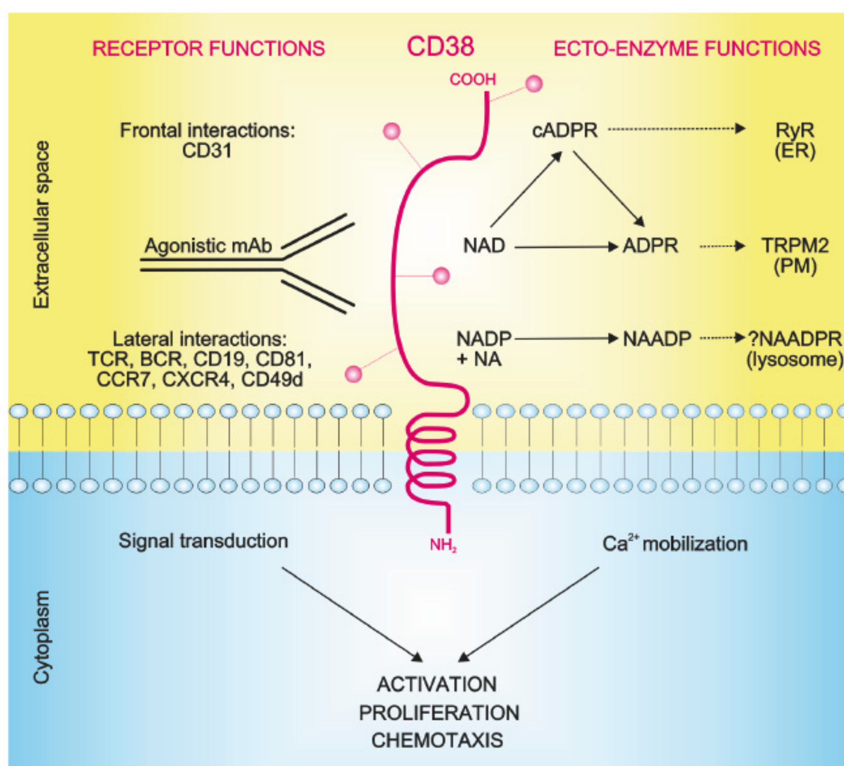

Fig. (1). CD38 at a glance. Schematic representation of the main structural and functional characteristics of human CD38. The molecule works as a receptor interacting frontally with the ligand CD31 and laterally with various surface molecules, resulting in large supramolecular complexes. These interactions are mimicked by agonistic mAbs and lead to the activation of an intracellular signal transduction cascade. CD38 also acts as a multifunctional ecto-enzyme, which converts NAD and NADP into cADPR, ADPR and NAADP (black arrows). The enzymatic products form powerful Ca^{2+}-mobilizing compounds inside the cell through binding ryanodine receptors, TRPM2 and an as yet unknown receptor located on lysosomal membrane (dotted arrows). The ensuing intracellular CD38-dependent signals modulate activation, proliferation and chemotaxis.

Abbreviations: ADPR, adenosine diphosphate ribose; cADPR, cyclic adenosine diphosphate ribose; NA, nicotinic acid; NAADP, nicotinic acid adenine dinucleotide phosphate; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; RyR, ryanodine receptor; TRPM2, transient receptor potential cation channel, subfamily M, member 2; ER, endoplasmic reticulum; PM, plasma membrane.

As an NAD-metabolizing enzyme, CD38 is part of a superfamily of enzymes that scavenge extracellular nucleotides by converting them to the related nucleoside [29]. Nucleotides such as ATP or NAD are released or leaked in the extracellular milieu by virtually every cell in the body, with increased levels being associated with stressful conditions [30]. However, strong evidence indicates that this intricate network of extracellular nucleotides/nucleosides, nucleotide-metabolizing enzymes and purinergic receptors serves multiple and more complex functions than mere scavenging [31]. Indeed, both nucleotides and nucleosides may bind to the multiple type-1 or -2 purinergic/pyrimidinergic receptors, acting per se as signaling molecules [32, 33]. Within the immune system, they are critical elements in the modulation of chemotaxis and in the articulated processes underlying immunoregulation.

A possible point of convergence between these two separate lines of research has emerged from studies on the crystal structure of CD38, which suggest that the active site of the enzyme might be turned on or off as a consequence of lateral and frontal interactions with other proteins [34]. This fine-tuning of the enzymatic activities would represent the latest evolutionary step for an enzyme that originated as a soluble protein with virtually endless degrees of freedom, but was subsequently limited by a number of constraints. Chronologically, the first would be the membrane anchorage, while the last would be represented by interactions with non-substrate ligands. These two constraints concur to define the human version of CD38.

THE CLL MODEL

CLL is defined as a proliferation of mature B lymphocytes expressing CD5 [35]. It is the most frequent adult leukemia in Europe and North America, with a median age of diagnosis ranging from 65 to 68 years old. The progressive accumulation of monoclonal B lymphocytes in the blood, bone marrow (BM), lymph nodes (LN) and spleen leads to leukocytosis, lymphadenopathy and splenomegaly. The functional consequences include BM failure with anemia, thrombocytopenia and recurrent infections. However, the clinical course of this still incurable disease is highly heterogeneous, ranging from a stable condition not requiring treatment to a rapidly progressive disease unresponsive to therapy [36].

The highly variable clinical phenotype of the disease is considered to be driven partly by the immunogenetic and molecular heterogeneity of the disease and partly by the host's genetic substrate [37]. The latter is reflected by the ability of the microenvironment to support and/or select CLL subclones with a more aggressive behavior [38]. In line with this view, studies on the clonal architecture of CLL have been very informative, supporting the conclusion that the average founder clone is admixed to small subclones, which may replace the older one under therapeutic or microenvironment-dependent selection pressures [39]. This "changing of the guards" is likely to take place in selected niches, located in the BM and/or the LN. Indeed, the malignant cells are dynamically compartmentalized into different districts, including blood, BM and LN, and modulate their growth potential and sensitivity to cytotoxic drugs in a site-dependent manner [40]. Only when located in the lymphoid organs, do CLL cells come into contact with the antigen and a cocktail of stimulatory and accessory signals that promote proliferation and survival [41]. These signals may also create genetic instability, with accumulation of novel mutations and/or expansion of previously existing mutated subclones. In both instances the outcome is disease progression. As a consequence of this network of proliferation/survival signals, CLL cells in the lymphoid niches are often shielded from the effects of chemotherapy, thus serving as a tumor reservoir from which relapse may occur [42, 43].

Research in this field has been very active in the past decade, focusing on the identification of i) prognostic markers and ii) therapeutic targets that hamper CLL homing to growth-favorable niches. CD38 may fulfill both these requisites.

CD38 AS A MARKER OF UNFAVORABLE PROGNOSIS FOR CLL PATIENTS

Several studies have demonstrated that expression of CD38 above a critical threshold is associated with an unfavorable outcome characterized by a shorter progression-free interval, earlier and more frequent treatment requirements and shorter overall survival [44-47]. The percentage of $CD38^+$ cells within a CLL clone is considered an indicator of the potential and actual degree of cellular activation of the clone. The threshold for positivity ranges from 7 to 30%, mostly depending on the antibody combination used. The current view is that CD38 expression defines a subpopulation of the clone that includes the proliferative compartment, as inferred by the presence in the same subset of the $Ki\text{-}67^+$ fraction and by a higher degree of telomerase activity compared to the negative counterpart [48-51]. Supportive data were obtained from analyses of CLL patients treated with heavy water (^2H_2O), which suggest that within individual clones there is a higher fraction of newly produced cells in the $CD38^+$ fraction than in the $CD38^-$ [52]. The finding of deleterious chromosomal abnormalities such as 11q and 17p deletions in patients with a $CD38^+$ clone is also consistent with an enhanced proliferative potential [53, 54] and a higher degree of clonal evolution [55-58]. Furthermore, $CD38^+$ cells display lower levels of the chemokine receptor CXCR4 than the counterpart, suggesting that they are actively recirculating to and from lymphoid organs [59]. Lastly, even if monoclonal in origin, the $CD38^+$ fraction of CLL clones was found to express a different set of genes, including some connected with vascular endothelial growth factor autocrine loop [60]. The conclusion from these experiments is that CD38 expression represents an indirect measure of cell division and reflects the *in vivo* growth of the neoplastic clone.

CD38 AS A DISEASE MODIFIER

Our initial involvement in the "CLL community" originated from an attempt to use CLL as a convenient model to derive information about the functions of CD38. Our working hypothesis is that CD38 is not merely a marker of a more activated status of the CLL cells, but rather a critical element in the pathogenetic network underlying disease maintenance and progression. This hypothesis was spurred by a number of observations that can be, for the sake of simplicity, divided

in two main themes: on the one hand, the role played by CD38 in the activation of neoplastic B cells and, on the other, its involvement in the modulation of the homing process of the leukemic clone.

CD38 Drives Proliferation of Leukemic Cells

To determine the functional role of CD38 in CLL cells we relied on a conventional approach, exposing $CD38^+$ CLL cells to mAb-mediated signaling. The results were initially disappointing with only a minority of cells showing detectable signals upon CD38 ligation. However, the situation was dramatically different when IL-2 was added to purified CLL cells: under these experimental conditions Ca^{2+} mobilization, proliferation and plasmablast differentiation of a subset of cells occurred [61]. This result was explained by hypothesizing that the dulled responses of the $CD38^+$ CLL cells to anti-CD38-mediated signaling were due to the low constitutive levels of surface expression of CD38. Indeed, IL-2 induced CD38 expression in time- and dose-dependent ways, likely allowing a signaling threshold to be reached. The ensuing working model was that CD38 signaling could be switched on or off as a result of the interactions between leukemic cells and the environment (represented by stromal cells and soluble factors). In this respect, the events recorded *in vitro* were considered to be a possible magnification of *in vivo* events occurring only in a minority of CLL patients undergoing Richter syndrome transformation. We then tried to mimic the LN environment *in vitro* by interacting $CD38^+$ CLL cells with nurse-like cells (NLC), a population of myeloid origin known to support CLL survival [62]. NLC expressed high levels of CD31: CD38/CD31 cross-talk significantly enhanced CLL growth and survival. Both events could be blocked using specific antibodies [63].

Attention was then turned to the molecular players and mechanisms underlying activation of the CD38 pathway. Our results indicated that a fundamental role is played by the cytoplasmic kinase ZAP-70, a further independent negative prognostic marker for the disease [64]. CD38 engagement in $CD38^+$/ZAP-70^+ CLL cells leads to a transient but significant tyrosine phosphorylation of ZAP-70. Further strengthening the relationship between these two players, the CD38 signaling pathway is selectively active in $CD38^+$/ZAP-70^+ cells, suggesting that ZAP-70 is a limiting factor for CD38-mediated functions [65]. Moreover gene expression data showed that the concomitant expression of these two molecules defines a subset of cells with a distinct genetic signature [66]. This information may offer a partial explanation to clinical observations indicating that the combined expression of CD38 and ZAP-70 more accurately identifies high-risk CLL patients [66, 67]. The final effects of this pathway are still an open issue. On the one hand, the two molecules may participate in the B cell receptor (BCR) signaling, as suggested by the higher responses to BCR signals in $CD38^+$ clones than in $CD38^-$ ones [68, 69] or by the activation of ZAP-70 following BCR engagement [70]. On the other hand, CD38 and ZAP-70 may be linked to the trafficking of CLL cells to lymphoid organs, which are sites of active proliferation. In line with this hypothesis, CD38 and ZAP-70 may work in association with chemokines/chemokine receptors and adhesion molecules that play an important role in controlling the ability of leukemic cells to recirculate from the blood to growth-permissive niches. Our subsequent studies were aimed at examining the latter possibility.

CD38 Regulates CLL Homing

An increasing body of evidence indicates that the host microenvironment critically supports growth, proliferation and survival of CLL cells. Within anatomically well-defined areas of the BM and LN, leukemic cells constantly interact with non-neoplastic cells: this cross-talk shapes the microenvironment and creates growth-supportive niches [43, 71]. Hence, the mechanisms regulating homing from blood to lymphoid organs are key elements in predicting tumor progression and resistance to therapy [37, 43, 72, 73]. They are also highly important to our understanding of the pathogenesis of the disease and developing of novel therapeutic strategies.

Data from gene expression profiling clearly indicate that the CD31/CD38 axis is of key relevance in this network and support the hypothesis that CD38 facilitates localization of leukemic cells in growth-permissive sites [74]. Indirect data supporting a role for CD38 in motility has derived from knock-out mouse models, where *Cd38*$^{-/-}$ leukocytes and dendritic cells display impaired ability to sense the chemokines present in the environment and to move in a gradient-dependent manner [75].

To confirm the hypothesis that CD38 has a compass-like function and guides CLL cells to growth-favorable niches, the homing process was broken down into distinct phases and the role of the molecule examined separately in each. The first phase marks the initiation of motility programs and is controlled by chemokines and their receptors, with a predominant role played by the CXCR4/CXCL12 receptor/ligand pair [76, 77]. During the second phase, which is mediated by integrins and their ligands, lymphocytes adhere to the endothelial barrier and negotiate crossing. The $\alpha 4$ integrin, also known as CD49d, is one of the main actors of this phase [78]: the fact that it is an independent negative prognostic marker for CLL patients adds a further, clinical perspective to this observation [79, 80]. The last phase is characterized by the production and secretion of matrix metalloproteases (MMPs) that allow lymphocytes to migrate within tissues and reach their final destination [81].

The emerging picture is that CD38 is a molecular facilitator that works in association with the main molecules involved in the control of these different steps. The first clue derives from analyses of chemotactic responses to the CXCL12, which showed that $CD38^+$ cells are more responsive than $CD38^-$ ones [82]. Support for this observation came studies of CLL patients with a bimodal expression of CD38: the negative component was significantly less responsive to the chemokine than the intact clone [82]. Moreover, ectopic expression of CD38 induced by genetic manipulation of primary $CD38^-$ cells was paralleled by increased functional responses to CXCL12, without any modification of CXCR4 expression.

Upon sensing of a chemokine gradient, leukemic cells need to bind to endothelial cells and to the extracellular matrix. These interactions lead to integrin outside-in activation [83], reorganization of the cytoskeleton and acquisition of

invasive properties. CD49d (the α4 subunit) regulates leukemic cell extravasation through binding to vascular cell adhesion molecule-1 (VCAM-1) or the CS1 fragment of fibronectin. Functional cooperation between CD49d and CD38 in regulating the adhesive properties of primary CLL cells has been the focus of recent studies: the presence of CD38 facilitates and enhances ability of leukemic cells to adhere to CD49d ligands. This synergy is regulated through the physical proximity and association of these molecules on the cell surface, as demonstrated by confocal microscopy analysis and biochemical experiments [84]. Moreover, engagement of CD38 by its non-substrate ligand CD31 is followed by the release of chemokines and cytokines by CLL cells, resulting in the up-regulation of VCAM-1 by stromal and endothelial cells and the activation of pro-survival signals in neoplastic cells [85]. These data provide further evidence for interaction between CD38 and CD49d and suggest a direct role in the pathogenesis of CLL.

The last step in the tumor invasion process is remodeling and degradation of the extracellular matrix [86]. CLL cells are able to produce and secrete MMP-9, a matrix metalloproteinase [87-89] whose expression is also correlated with more aggressive progression of the disease [90]. In leukemic cells, engagement of CXCR4 or CD49d leads increased secretion of MMP-9, through the activation of specific signaling pathways [89]. Investigation of a large cohort of patients shows that CD38$^+$ cells are characterized by higher levels of MMP-9 than negative ones. Moreover, the engagement of CD38 by agonistic monoclonal antibodies results in an upmodulation of the gelatinase [91].

The emeriging picture is that CD38 may functionally associate with several molecules, modulating their effects and controlling the ability of leukemic cells to migrate and home to specific microenvironment, where its ability to perform as a receptor can enhance the proliferative potential of CLL cells (Fig. **2**).

IN VIVO MODELS OF CLL AS TOOLS TO INVESTIGATE CD38 FUNCTIONS

Most of what we know about the molecular features and biological processes characterizing CLL cells and have observed about the impact of novel therapeutic drugs derives from *in vitro* experiments using primary leukemic cells and few existing cell lines. Until recently animal models of CLL were unavailable mainly because of the lack of known specific genetic lesions. The generation of a transgenic mouse overexpressing the human *TCL1* gene under the control of the immunoglobulin heavy chain variable region promoter and immunoglobulin heavy chain enhancer (Eμ-TCL1) provided initial solutions to this problem. At the age of about 13-18 months these mice develop a lymphoproliferative disease resembling human CLL, characterized by peripheral-blood lymphocytosis, splenomegaly, lymphoadenopathy and infiltration of neoplastic lymphocytes in the liver, kidney and lungs [92]. However, despite its continuing usefulness for *in vivo* testing the efficacy of novel drugs or drug combinations [93], the model has serious limitations. The first is the long period of time it takes for the disease to develop and the obvious practical difficulties associated to this waiting period. The second, and perhaps most relevant one, is that the devel-

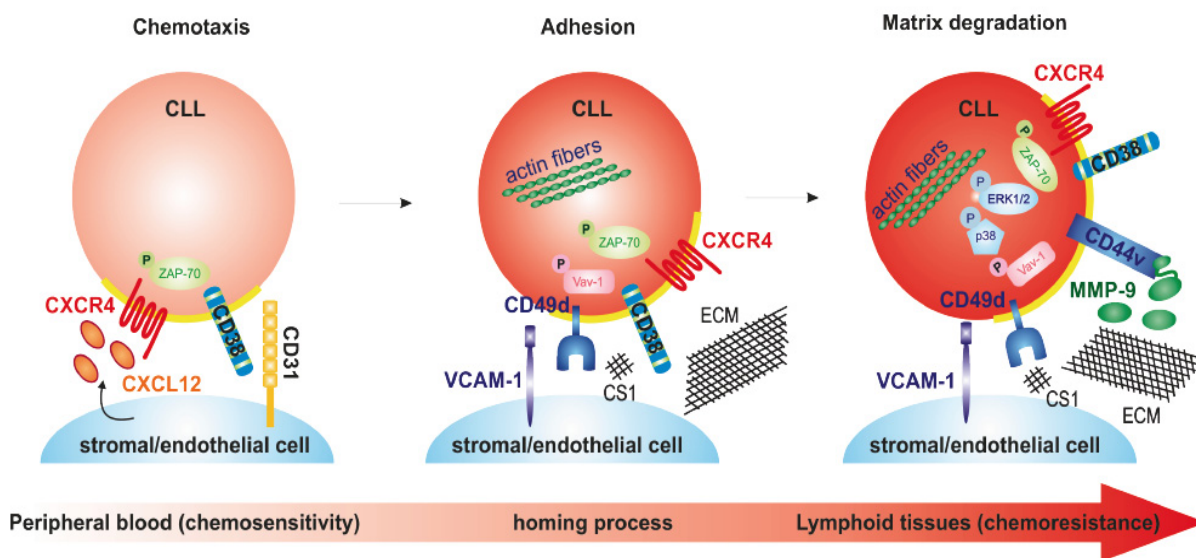

Fig. (2). CD38 as a disease modifier in CLL. Once expressed by a CLL cell, CD38 acts as a facilitator of the homing process. CD38/CD31 signals enhance chemotactic responses to CXCL12, CD49d-mediated adhesion to VCAM-1 or the CS1 fibronectin fragment and MMP-9 secretion, responsible for extracellular matrix (ECM) degradation. This is achieved by recruiting a molecular platform (indicated by the yellow line) that connects surface molecules with the intracellular signaling machinery. The main components have been identified as ZAP-70 and Vav-1, converging on the MAP-kinase family. As a consequence, CD38$^+$ CLL cells recirculate from blood to lymphoid organs more efficiently than CD38$^-$ ones. Localization in growth-favorable niches may potentiate proliferative responses, likely contributing to disease progression and increased resistance to conventional chemotherapy. Due to its role in the homing process, CD38 represents an attractive therapeutic target. Specific monoclonal antibodies or selective inhibitors of the enzymatic activity may help trap leukemic cells in the blood stream, rendering them more susceptible to chemotherapeutic regimens.

oping leukemias invariably overexpress the *TCL1* gene as a pathogenetic mechanism and thus do not fully recapitulate the human disease, which is caused by a plethora of genetic lesions.

These limitations can be at least partially overcome by exploiting xenograft models based on the transplantation of human primary cells or human cell lines in immunodeficient mice. The efficiency of these animal models has significantly improved owing to the introduction of more severely immunocompromised animals that lack the ability to reject xenogeneic cells. Intravenous injection of human CLL cells in NOD/SCID mice leads to splenic engraftment, with the number of engrafted cells reflecting the aggressiveness of the human disease [94]. In a recent study examining the efficiency of engraftment and growth of molecularly distinct CLL clones, $CD38^+$ CLL clones had better engraftment ability than the $CD38^-$ leukemic cells. Furthermore, $CD38^+$ CLL cells *in vivo* were more highly proliferative, as shown by Ki-67 staining [95]. The same model was also used in short-term homing experiments and demonstrated that $CD38^+$ CLL cells migrate more efficiently to spleen and BM than their negative counterparts [95]. This is in agreement with previous evidence obtained *in vitro* indicating the enhanced ability of $CD38^+$ CLL cells to migrate towards CXCL12, thereby indirectly validating both models [82].

Engraftment of human cells can be further improved by using more severely compromised animal models, such as the NOD/SCID/γ $chain^{-/-}$ (NSG) mice. The conditioning of the mouse environment with human "supportive" cells betters the outcome and underlines the critical role of BM stromal cells and of T cells of CD4 origin. This can be done by transferring human cord blood-derived $CD34^+$ and BM-derived stromal cells to condition the mouse environment before engrafting CLL cells [96]. Engraftment potential can also be improved by adding soluble factors inducing immunomodulation also enhances [97].

We have exploited the latter models in preliminary homing experiments that underline the importance of CD38 in guiding CLL cells out of the blood and into the BM and spleen. Pre-treatment of neoplastic cells with blocking anti-CD38 monoclonal antibodies diminished ability of CLL cells to home to spleen and BM, trapping them in the peripheral blood [82]. Future studies should be undertaken to confirm and expand these results in larger cohorts. For now, they represent an initial pre-clinical indication of the potential relevance of CD38 as a therapeutic target for selected subsets of patients.

CD38 AS A THERAPEUTIC TARGET?

Targeted immunotherapy with human mAbs as drugs has been highly successful in many forms of cancer. This is exemplified by rituximab, a chimeric anti-CD20 mAb, which revolutionized the treatment of several B-cell malignancies. The recent announcement of a $1.1 billion deal between Johnson & Johnson and Genmab to license an anti-CD38 monoclonal antibody (daratumumab) [98, 99] testifies in favor of a keen interest of the hematological community on the topic. The antibody is in early-stage development for multiple myeloma (MM) and for selected cases of CLL. Preclinical data showed that daratumumab not only kills MM cells via complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and apoptosis *in vitro*, but also inhibits growth of these tumor cells in different xenograft models [100, 101]. Moreover this antibody would be suitable for drug combination regimens, with novel chemotherapeutics and immunomodulatory agents such as lenalidomide [102]. Two other anti-CD38 mAbs are also in early-stage trials: SAR650984, a humanized mAb developed by Sanofi and MOR03087, a human mAb developed by MorphoSys. A note of caution needs to be made regarding the potential side effects of an anti-CD38-based mAb therapy, due to the molecule's widespread distribution even outside of the immune system [103].

Because CD38 is a key element in the pathogenetic network underlying CLL, drugs inhibiting the activity of the molecule might have potential therapeutic implications also for this disease. Beside monoclonal antibodies, one approach would be to use inhibitors of the enzymatic activity. Preliminary data from recent studies indicates that CD38 might be inhibited, at least *in vitro*, by flavonoids [104]. These natural molecules belong to a vast group of polyphenolic compounds widely found in all foods of plant origin and known as plant pigments for over a century [105]. Interest in the possible health benefits of polyphenols (particularly flavonoids) is based on their antioxidant and free-radical scavenging abilities, mostly observed *in vitro*. Among them luteolinidin, kuromanin and luteolin are potent inhibitors of CD38 enzymatic activities [104]. Other two flavonoids, quercetin and apigenin, are also able to block CD38 activities, [106] and have been shown to have beneficial effects in cancer models [107-109].

This evidence suggests that inhibition of CD38 may be successful as a novel therapeutic strategy to treat not only selected forms of cancer, but also non-tumoral diseases in which CD38 is highly expressed and has a pathogenetic role. The next decade will likely tell whether these promises are actually fulfilled.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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