Leukemia & Lymphoma, 2011; Early Online, 1–9

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REVIEW

The secret second life of an innocent chaperone: the story of CD74 and B cell/chronic lymphocytic leukemia cell survival

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(Received 3 February 2011; revised 18 February 2011; accepted 19 February 2011)

Abstract

This review deals with the cytokine macrophage migration inhibitory factor (MIF) and its receptor, CD74. MIF and CD74 have been shown to regulate peripheral B cell survival and were associated with tumor progression and metastasis. CD74 expression has been suggested to serve as a prognostic factor in many cancers, with higher relative expression of CD74 behaving as a marker of tumor progression. In chronic lymphocytic leukemia (CLL) cells, binding of MIF to CD74 induces nuclear factor-κB (NF-κB) activation and up-regulation of TAp63 expression, resulting in the secretion of interleukin 8 (IL-8), which in turn promotes cell survival. In addition, TAp63 expression elevates expression of the integrin VLA-4, particularly during the advanced stage of the disease. Blocking of CD74, TAp63, or VLA-4 inhibits the *in vivo* homing of CLL cells to the BM. Thus, CD74 and its target genes, TAp63 and VLA-4, facilitate migration of CLL cells back to the BM, where they interact with the supportive BM environment that helps rescue them from apoptosis. These results are expected to pave the way toward novel therapeutic strategies aimed at interrupting this survival pathway. One such agent, the monocolonal antibody milatuzumab directed at CD74, is already being studied in early clinical trials.

Keywords: *Development of B lymphocytes, cell cycle and apoptosis changes, lymphoid leukemia*

Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL), the most common Western adult leukemia, is characterized by the progressive accumulation of small mature CD5+ lymphocytes in the peripheral blood, lymphoid organs, and bone marrow (BM). The main feature of the disease is decreased apoptosis, resulting in the pathologic accumulation of these malignant cells [1], although other processes such as cell proliferation and clonal evolution are also involved [2]. Despite major progress in the last few years in the understanding of the biology and pathophysiology of this disease, as well as the development of better treatment modalities, CLL remains incurable in most patients who require treatment, and even control of the disease requires aggressive treatment, with significant side effects.

The complex processes involved in proliferation and survival of CLL cells are beyond the scope of this review, and have been discussed at length in a review by Caligaris-Cappio and Ghia [2]. Briefly, CLL cell survival is regulated by intracellular signaling pathways that are activated by various stimuli from the microenvironment. Several lines of evidence suggest that the survival advantage of CLL lymphocytes may be due to the induction of anti-apoptotic proteins of the Bcl-2 family [3]. High levels of Mcl-1 and Bcl-2 mRNA [3] and protein [4] have been found in CLL. These proteins act by binding pro-apoptotic proteins to prevent them from disrupting the mitochondrial outer membrane potential. In addition, it is clear that deletion of p53 has a significant role in progression, lack of response to treatment, and the aggressive course of CLL.

Among the signals that regulate the survival of CLL cells are cascades that were previously shown to regulate survival of normal peripheral B cells. Antigenic stimuli that are propagated through the B-cell receptor (BCR) play a key role in the initiation,

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ISSN 1042-8194 print/ISSN 1029-2403 online © 2011 Informa UK, Ltd.
DOI: 10.3109/10428194.2011.565437

maintenance, proliferation, and evolution of the malignant clone [5–8]. An additional survival signal in CLL is mediated by a member of the tumor necrosis factor family, known as B cell-activating factor of tumor necrosis factor family (BAFF) [9]. Interaction of BAFF with its receptors (B cell maturation antigen [BCMA], transmembrane activator and calcium modulator and cytophilin ligand interactor [TACI], and BAFFR) can enhance leukemia cell survival *in vitro*, a mechanism that could potentially contribute to disease progression *in vivo* [10]. This review will discuss a novel survival mechanism that was recently shown to control follicular B cell and CLL cell survival, regulated by the cytokine macrophage migration inhibitory factor (MIF) and its receptor CD74, and the potential of this pathway as a therapeutic target in this disease.

Macrophage migration inhibitory factor (MIF)

MIF accounts for one of the first cytokine activities to have been described [11]. MIF was originally identified as a T-cell-derived factor responsible for the inhibition of macrophage migration [12]. The crystal structure of MIF revealed the active form to be a 37.5 kDa homotrimer with novel protein folds that defined a previously unknown structural superfamily [13,14]. In addition to its distinctive structure, MIF possesses a unique tautomerase enzymatic activity, revealed through its structural homology to several bacterial enzymes. This activity mediated by an N-terminal proline residue allows MIF to catalyze the conversion of non-physiological substrates such as D-dopachrome or L-dopachrome methyl esters to their indole derivatives. As yet, no human physiological substrates for MIF tautomerase have been identified [15].

Extensive studies have revealed the central role of MIF, secreted from almost all type of cells, in innate and adaptive immunity. MIF promotes monocyte/macrophage activation and is required for the optimal expression of tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), and prostaglandin E_2 (PGE_2) [16–18]. The role of MIF in adaptive immunity is less fully characterized, but antibody-based neutralization of MIF inhibits delayed-type hypersensitivity, T cell priming, and antibody production *in vivo* [19,20]. It was also shown that MIF recognizes a cell surface receptor, the CD74 extracellular domain, resulting in the initiation of a signaling pathway [21].

In addition, MIF has been associated with tumor progression. It was reported that MIF mRNA is overexpressed in various tumors, and MIF has also been associated with the growth of malignant cells. MIF was shown to be overexpressed in solid tumors [22], and it is frequently overexpressed in primary breast cancer tissues, where it plays a role in tumor–stroma interactions of primary breast cancers [23]. MIF is also associated with tumor growth and tumor-associated angiogenesis in a murine colon cancer cell line [24]. In addition, anti-MIF immunoglobulin (Ig) therapy has been shown to suppress tumor growth [25].

CD74

CD74 is a non-polymorphic type II integral membrane protein that is expressed on antigen-presenting cells. The CD74 chain was initially thought to function mainly as a major histocompatibility (MHC) class II chaperone [26]. MHC class II molecules are heterodimeric complexes that present foreign antigenic peptides on the cell surface of antigen-presenting cells (APCs) to CD4+ T cells [27–29].

MHC class II synthesis and assembly begins in the endoplasmic reticulum (ER) with the non-covalent association of the MHC α and β chains with trimers of CD74. Three MHC class II αβ dimers bind sequentially to a trimer of the CD74 to form a nonameric complex $(\alpha\beta CD74)_3$, which then exits the ER [30]. After being transported to the *trans*-Golgi, the αβCD74 complex is diverted from the secretory pathway to the endocytic system compartments. In the endocytic compartments, CD74 is gradually proteolytically processed, leaving only a small fragment, the class II-associated Ii chain peptide (CLIP), bound to the released αβ dimers. The final step for MHC class II expression requires interaction of αβCLIP complexes with another class II-related αβ dimer, called human leukocyte antigen (HLA)-DM in the human system, and H2-M in mice. Binding of this molecule drives out the residual CLIP peptide, rendering the αβ dimers ultimately competent to bind antigenic peptides, which are mainly derived from internalized antigens and are also delivered to the endocytic pathway [31,32]. The peptide-loaded class II molecules then leave this compartment, by an unknown route, to be expressed on the cell surface and surveyed by CD4+ T cells.

A small proportion of CD74 is modified by the addition of chondroitin sulfate (CD74-CS), and this form of CD74 is expressed on the surface. The cell surface CD74 molecule serves as a receptor in many types of cells and can initiate various signaling cascades. CD74 was reported to be a high-affinity receptor for MIF [21]. *Helicobacter pylori* was shown to bind to CD74 on gastric epithelial cells and to thereby stimulate IL-8 production [33]. In addition, it was shown that in mice lacking CD74, there is an accumulation of transitional I (TI) B cells in the

periphery, characterized by low expression levels of IgD and CD23 and poor response to T-independent antigens [34–37], while the mature population responsible for the humoral immune response is missing [34,38].

An additional cell surface molecule, CD44, was described as an integral component of the CD74 receptor complex [39,40]. While CD74 is sufficient for the binding of MIF to the cell surface, CD44 was found to be necessary for MIF signal transduction in these cells [40]. CD44 is a broadly expressed single-pass transmembrane protein with known kinase-activating properties.

Associations have been described between CD74 expression/activation and tumor development and progression. The overexpression of CD74 was observed in various cancers [39,41–45] including CLL [46,47]. CD74 expression in solid tumors has been suggested to serve as a prognostic factor, with higher relative expression of CD74 behaving as a marker of disease progression [48]. CD74 has also been implicated in the pathogenesis of metastasis [49]. Moreover, a humanized anti-CD74 monoclonal antibody (mAb) (milatuzumab; hLL1: see below) was shown to have therapeutic activity in a xenograft model of multiple myeloma, perhaps by inhibiting the high level of CD74 expression in this plasma cell malignancy [50].

Macrophage migration inhibitory factor regulates normal B-cell survival in a CD74-dependent manner

B cells develop in the bone marrow and, through a process that involves rearrangement and expression of Ig genes, produce an antigen-specific receptor, which is first manifested in the immature stage. Immature B cells emerge from the bone marrow (BM) to the periphery and migrate to the spleen for their final maturation step [51]. However, in the steady state, up to a quarter of the mature IgD+ IgM+ B pool can subsequently be found recirculating through the BM [52]. During their development, B cells encounter various checkpoints that control cell survival. Under steady state conditions, the number and distribution of B cells are under homeostatic control due to a balance between survival and apoptosis.

A complex of CD74 and CD44 is essential for initiating the signaling cascade induced by macrophage migration inhibitory factor. Our studies have shown that CD74 expressed on B cells is directly involved in shaping the B-cell repertoire by regulating murine mature B-cell survival [34,38,53] through a pathway leading to the activation of transcription mediated by the nuclear factor-(B (NF-(B) p65/RelA homodimer and its co-activator, TAFII105 [54]. NF-(B activation is mediated by the cytosolic region of CD74 (CD74-ICD), which is liberated from the membrane and translocates to the nucleus [55]. It was shown that arrival to the endocytic compartments and a series of proteolytic cleavages within these compartments are necessary for the release of CD74-ICD from the membrane [56]. The removal of the CD74 lumenal domain is the first step required for release of the N-terminal cytosolic fragment leading to NF-κB activation.

To follow the CD74 downstream cascade, cell surface CD74 was stimulated in B cells with agonistic anti-CD74 antibody or MIF. CD74 stimulation was shown to induce a signaling pathway that involves Syk tyrosine kinase and the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. In addition, stimulation of CD74 induces CD74 intramembrane cleavage and the release of CD74-ICD. CD74-ICD translocates to the nucleus where it regulates transcription of genes that control B cell proliferation and survival [57,58].

In B cells, CD74 forms a complex with CD44 that is essential for the MIF-induced signaling cascade [58]. The complex is formed even in the absence of MIF, since no change is detected in the formation of the complex or in CD44 expression following MIF stimulation. Nevertheless, formation of the CD74/CD44 complex was shown to be crucial for the signaling cascade induced by MIF [58]. MIF was found to induce cell entry into the S-phase in a CD74- and CD44-dependent fashion by elevating cyclin E levels, resulting in cell proliferation. In addition, this cascade elevates Bcl-2 expression, supporting cell survival [57,58]. Thus, MIF binding to both CD74 and CD44 initiates a survival pathway, resulting in proliferation of the mature B-cell population, and their rescue from death.

The mammalian BM is the major site of adult hematopoiesis. Importantly, the recent advent of advanced imaging studies has led to the identification of unique niches that provide a highly specialized microenvironment for distinct developmental processes. These include anatomically defined niches for hematopoietic stem cells [59,60], and for B cell development [61].

MIF secreted from BM dendritic cells regulate mature B cell maintenance

The BM harbors dendritic cells (bmDCs) that function as myeloid BM cells and display an activated phenotype. Most intriguingly, these cells are concentrated into unique perivascular clusters that wrap a distinct set of sinusoids and venules [62]. Conditional ablation of bmDCs results in the specific

loss of both endogenous and adoptively transferred mature B cells from the BM immune niches. This failure of bmDC-depleted BM to support B cell engraftment could be overcome by the overexpression of the anti-apoptotic factor, Bcl-2, in the mature B cells, suggesting that bmDCs provide a unique survival factor. Studies using mixed BM chimeras subsequently showed that this factor is MIF. Thus, mature B-cell maintenance requires MIF-producing bmDCs [62]. Newly formed mature B cells emerge from the spleen and circulate in the body. In the BM, a survival signal induced by MIF and secreted from bmDCs is essential for B cell maintenance.

CD74 was also found to be expressed on normal colon epithelial cells (CECs). Similar to B cells, stimulation of CD74 on CECs by MIF, which is expressed throughout the human gastrointestinal tract, induces a signaling cascade leading to up-regulation of Bcl-2 and cyclin E expression, resulting in a significant increased survival of these cells in a CD44-dependent manner [63].

c-Met and its ligand hepatocyte growth factor regulate mature B-cell survival in a pathway induced by macrophage migration inhibitory factor binding to CD74/CD44. Cell surface receptor CD44 has been implicated in regulation of the activation of the tyrosine kinase receptor, c-Met [64–66], although the precise mechanism of their interaction is unknown.

c-Met is a unique disulfide-linked $\alpha\beta$ heterodimeric receptor tyrosine kinase with a versatile role in regulating numerous biological functions in response to its natural ligand, hepatocyte growth factor/scatter factor (HGF). HGF is a multifunctional cytokine with a domain structure and proteolytic mechanism of activation similar to that of the serine protease plasminogen. Activation of the HGF/c-Met signaling pathway, which requires phosphorylation of various specific tyrosine residues on c-Met itself, leads to cellular responses, including increased motility, proliferation, morphogenesis, and cell survival [67–73].

Following MIF stimulation, c-Met engages with CD74 and CD44 on the cell membrane and, together with HGF, triggers an additional signaling pathway, which is necessary to initiate the MIF-induced survival signaling cascade [74]. The HGF-induced survival pathway controls proliferation and survival of peripheral B cell subsets. HGF enhances the survival of the mature B-cell population in the spleen, whereas there is no change in the cell death of the immature population. The CD74/CD44 cell surface complex regulates the HGF/c-Met-induced survival cascade, as, in cells lacking CD74, exogenous HGF is able to bypass the absent survival signal and rescue the mature B-cell population that is missing in these cells. Moreover, blocking HGF or c-Met activity abolishes MIF-induced Syk phosphorylation and Bcl-2 elevation, thereby inhibiting cell survival [74]. The precise mechanism by which MIF activates c-Met is still unclear. However, since HGF was shown to be sufficient to support survival of mature B cells, and its blockade inhibits the MIF-induced survival pathway, it is believed that HGF is involved in the MIF-induced survival cascade [74].

Thus, c-Met participates in controlling MIF-induced signaling by forming a survival complex together with CD74 and CD44 in B cells. These findings establish a key role for the HGF/c-Met pair in the regulation of B-cell survival, demonstrating an additional level of control of the humoral immune response.

Macrophage migration inhibitory factor /CD74 induces TAp63 expression, which regulates B cell survival. Stimulation by MIF was shown to induce the activation of the p65/RelA member of the NF-κB family, which in turn up-regulates TAp63 transcription and expression [75].

The p63 gene exhibits high sequence and structural homology to p53 [76]. Like p53, the p63 gene encodes an N-terminal transactivation domain, a core DNA binding domain, and a carboxy-terminal oligomerization domain. The p63 gene contains two transcriptional start sites that are used to generate transcripts encoding proteins with or without an N-terminal transactivation domain. Proteins with the transactivation domain are termed TAp63, and proteins lacking the transactivation domain are known as ΔNp63. p63 was shown to play a role in developmental regulation of limbs, skin, most epithelial tissues, and epidermal differentiation [77,78].

In B cells, TAp63 exhibits a behavior favoring cell survival. TAp63 binds to the Bcl-2 promoter and induces the transcription of Bcl-2 mRNA, and production of the Bcl-2 anti-apoptotic protein, which enhances cell survival. Thus, the MIF/CD74/NF-κB/TAp63 axis defines a novel anti-apoptotic pathway in mature B cells, shaping both the B cell repertoire and the immune response [75].

Macrophage migration inhibitory factor and CD74 in autoimmunity

MIF has been implicated in the pathogenesis of numerous inflammatory and autoimmune disorders [79]. Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that is characterized by the loss of immune tolerance and the production of autoantibodies to nucleic acids and nucleoproteins [80]. The immunopathology associated with SLE results primarily from immune complex deposition in the small vessels of the skin, kidney, and other organs; this leads to the activation of complement and Ig Fc receptors and the recruit-

ment of neutrophils and monocytes. In addition, SLE is characterized by impaired B-cell and T-cell functions and is associated with serological and clinical manifestations that involve multiple organ systems and abnormal B-cell activation and differentiation [80].

It was recently shown that B lymphocytes from SLE-afflicted mice express elevated levels of CD74, compared with B cells from healthy mice [81]. Two lupus-prone mouse strains manifest a time-dependent elevation in circulating MIF at ages that correspond with disease progression and the development of glomerulonephritis [82]. MIF [82], CD74, and CD44 [81] mRNA and protein expression in kidneys are also increased significantly in parallel to inflammatory progression in lupus-prone mice. In B cells from the diseased mice, MIF expression is also up-regulated [81]. For the specific treatment of SLE, a peptide designated hCDR1, which is based on the sequence of the complementarity-determining region (CDR)- 1 of an autoantibody [83], was designed and shown to ameliorate lupus manifestations in both spontaneous and induced models of SLE [84,85]. Induction of the MIF/CD74 pathways in B cells of SLE-diseased mice is associated with their increased survival. Treatment with hCDR1 diminishes the expression of CD74 and CD44 molecules to the levels generally detected in young healthy mice, resulting in reduced B-cell survival [81]. Furthermore, treatment with (*S*,*R*)-3-(4-hydroxyphenyl)- 4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1), a MIF antagonist, reduces the MIF-dependent proinflammatory cytokine production and leukocyte recruitment and ameliorates immune-mediated renal injury [82]. These results suggest that the CD74/MIF pathway plays an important role in survival of pathogenic B cells and in lupus pathology.

Macrophage migration inhibitory factor and CD74 in chronic lymphocytic leukemia

MIF and CD74 in CLL cell survival

CD74 and its ligand, MIF, which have a significant role both in autoimmunity and in tumor progression, were shown to play a pivotal role in the regulation of CLL cell survival [86]. CLL cells markedly up-regulate both expression of their cell surface CD74, and their MIF production. Although overexpression of CD74 in CLL has been previously shown, the role of CD74, which was then considered to be simply a chaperone molecule of the MHC-II system, in the pathogenesis of the disease was not clear. We found that stimulation of CD74 with the MIF ligand (as well as with an agonistic antibody) initiates a signaling cascade leading to IL-8 transcription and secretion in all CLL cells, regardless of the clinical status of the patients. Secreted IL-8 induces the transcription and translation of the anti-apoptotic protein, Bcl-2, and thus regulates an anti-apoptotic pathway, though no effect on proliferation is observed. Blocking of CD74 (by milatuzumab), or of MIF or IL-8, results in dramatic down-regulation of Bcl-2 expression, and augmentation of apoptosis [86].

IL-8 is a member of the CXC chemokine family, which plays an important role in autoimmune, inflammatory, and infectious diseases [87–89]. Because of its potent proinflammatory properties, IL-8 is tightly regulated, and its expression is low or undetectable in normal tissues. However, it is now known that IL-8 also possesses tumorigenic and pro-angiogenic properties [90]. Increased serum levels of IL-8 have negative prognostic significance in CLL [91], lending further support to the importance of this pathway in the pathogenesis and progression of disease. The signaling cascade induced by CD74, which activates NF-κB, results in increased IL-8 expression and in an autocrine/paracrine survival response (Figure 1). Thus, in CLL, MIF and CD74 induce an important survival mechanism, which appears to operate from the very early stages of the disease [86].

In addition, blocking CD74 using humanized anti-CD74 blocking antibody (milatuzumab) specifically down-regulates TAp63 expression levels, were resulting in a specific inhibition of the MIF-induced elevation of Bcl-2 expression levels in CLL cells, further demonstrating that the MIF/CD74-induced survival cascade is mediated through TAp63 [92].

MIF and CD74 regulate CLL cell homing to the BM

The BM stroma plays an essential role in B-lymphopoiesis, and can provide survival niches for both normal and leukemic mature B cells. The adhesion of CLL cells to BM stromal cells or to the BM vasculature has been shown to rescue these lymphocytes from apoptosis and to extend their life span (62,93,94). The increased accumulation of CLL cells in the BM during disease progression suggests a change in the migratory and homing pattern of the cells. Advanced stage CLL cells express higher levels of the VLA-4 integrin compared to early stage cells [92,95–97].

MIF and CD74 were demonstrated to play a significant role in the regulation of VLA-4 expression, and therefore to affect homing and survival of CLL cells [92]. MIF is secreted from almost all types of cells; therefore, CLL cells are stimulated by this chemokine in all compartments. MIF stimulation

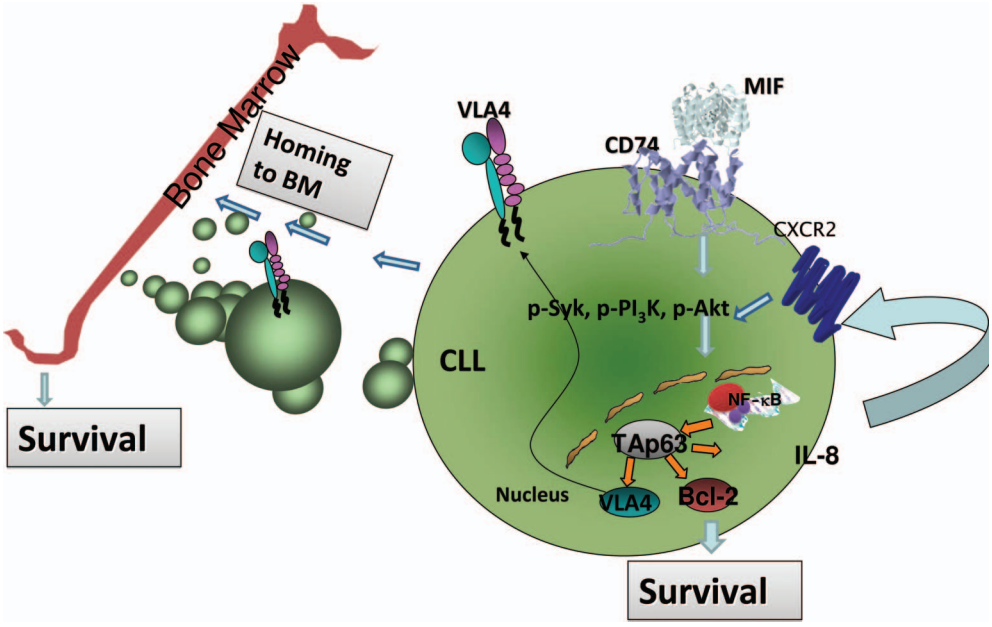

Figure 1. Schematic representation of the dual role of MIF-induced regulation in CLL survival affecting cell survival both directly (by elevating Bcl-2 expression) and indirectly (by elevating VLA-4 expression, promoting homing to the BM).

elevates VLA-4 cell surface expression levels during advanced stage disease. It is likely that only after their progress to the advanced disease stage do the cells increase their VLA-4 expression to levels that support their homing to the BM, though the mechanism of VLA-4 regulation is not known. Thus, homing to the BM requires threshold levels of VLA-4 expression that enable retention and survival of CLL in BM, an environment which is enriched with the VLA-4 ligands, VCAM-1, and fibronectin, and further supports their retention and survival. It is possible that CLL exposure to systemic MIF redirects circulating CLL cells back to the BM, where they may encounter more MIF, and further elevate their VLA-4 expression and retention on stromal VLA-4 ligands. The VLA-4–finbronectin interaction has been shown to have a significant effect on CLL cell survival [98], as well as a protective effect against fludarabine-induced cell death [99]. This may create a cycle that can promote disease-associated bone marrow failure.

These results suggest that blocking MIF expression or its receptor, such as with an antagonistic anti-CD74 antibody, might inhibit survival of CLL cells and their homing to the BM, (Figure 1). Thus, novel therapeutic strategies aimed at blocking the MIF/CD74 pathway either alone or in combination with other chemo- or immunotherapuetic agents could lead to enhanced and better targeted eradication of the disease due to decreased cell survival, and/or alteration of disease progression by decreasing bone marrow homing, and occupation of normal hemato-poietic niches.

CD74 as a potential therapeutic target for lymphoid malignancies

Milatuzumab (Immunomedics) is a novel humanized monoclonal antibody targeting CD74. It exhibits selective binding and rapid internalization into CD74-positive cancer cells. Milatuzumab with or without conjugated toxins or other chemotherapeutic agents elicits significant anti-tumor effects in xeno-graft models of various lymphoid malignancies in mice. This effect is also synergistic with other mAbs such as rituximab [100]. In a phase I clinical trial, milatuzumab showed no severe adverse effects in patients with relapsed/refractory multiple myeloma, and it stabilized the disease in some patients for up to 12 weeks [101]. Ongoing trials testing different treatment schedules of milatuzumab in chronic lymphocytic leukemia, non-Hodgkin lymphoma, and multiple myeloma indicate that milatuzumab is free of severe adverse effects in humans, and can probably be safely administered with other agents. The combination of milatuzumab with the anti-CD20 mAb velatuzumab is now being tested in patients with refractory non-Hodgkin lymphoma. A recent study also showed that incorporation of milatuzuamb into liposomes further enhances its therapeutic potential in CLL [102].

Our preliminary clinical results in CLL suggest that milatuzumab is well tolerated for prolonged periods (6 months and more) even in frail elderly patients, with other co-morbidities, and has a mild inhibitory effect on disease when given as a single agent, even in patients with adverse prognostic

features who have failed other modalities of treatment. This suggests its use as a single agent at least in some of those patients whose functional status makes them ineligible for other more aggressive forms of treatment. Possibly, milatuzumab could be used as a more effective treatment in combination with low doses of fludarabine, as blocking the CD74 pathway could potentially overcome the protective effect of fibronectin via VLA-4 [99]. Further studies are required in order to fully uncover the optimal mode, timing of administration, and therapeutic potential of milatuzumab in CLL as well as other lymphoid malignancies.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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