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► **To cite this version:**

Sofiane Fodil, Marine Arnaud, Camille Vaganay, Alexandre Puissant, Etienne Lengline, et al.. Endothelial cells: major players in acute myeloid leukaemia. *Blood Reviews*, 2022, 54, pp.100932. 10.1016/j.blre.2022.100932 . hal-03854294

HAL Id: hal-03854294

<https://cnrs.hal.science/hal-03854294v1>

Submitted on 22 Jul 2024

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Title: Endothelial cells: major players in acute myeloid leukaemia

Sofiane Fodil^{a,b}, Marine Arnaud^b, Camille Vaganay^c, Alexandre Puissant^c, Etienne Lengline^d,
Nuala Mooney^b, Raphael Itzykson^{c,d} and Lara Zafrani^{a,b,*}

^a : Medical Intensive Care Unit, Saint Louis Hospital , Assistance Publique des Hôpitaux de Paris, University of Paris, Paris, France

^b : University of Paris, Immunologie Humaine, Pathophysiologie, Immunothérapie UMR 976, INSERM, Paris, France

^c : University of Paris, Génomes, biologie cellulaire et thérapeutique U944, INSERM, CNRS, F-75010, Paris, France

^d : Adult Hematology Unit, , Assistance Publique des Hôpitaux de Paris, University of Paris, Paris, France

*Corresponding author : Pr Lara Zafrani, Medical Intensive Care Unit, Hopital Saint-Louis, Assistance Publique des Hôpitaux de Paris, University of Paris, 1 avenue claude vellefaux, 75010 Paris, France, email : lara.zafrani@aphp.fr

Co-author email address:

sofiane.fodil@aphp.fr

marine.arnaud@inserm.fr

camille.vaganay@inserm.fr

alexandre.puissant@inserm.fr

etienne.lengline@aphp.fr

nuala.mooney@paris7.jussieu.fr

raphael.itzykson@aphp.fr

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Abstract

The role of the vascular microenvironment is increasingly studied in acute myeloid leukaemia (AML). Complex interactions between endothelial cells (ECs) and pre-leukaemic cells may contribute to the clonal evolution of pre-leukaemic stem cells in the bone marrow niche and to the proliferation, survival and chemoresistance of leukaemic cells. Through the expression of different adhesion molecules, ECs play a key role in the development of specific acute complications of AML, including leukostasis, acute respiratory failure, acute kidney injury or neurological complications. Moreover, in newly diagnosed patients, leukaemic cells promote endothelial activation and subsequent disseminated intravascular coagulation. Mechanisms of this bi-directional dialogue between leukaemic cells and ECs will reveal possible therapeutic targets to be explored to improve the survival of AML patients.

Keywords

Acute myeloid leukaemia, endothelial cell, hematopoietic stem cell

1. Introduction

Acute myeloid leukaemias (AML) are oligoclonal neoplasms resulting from the multistep acquisition of somatic genetic and epigenetic alterations in a hematopoietic stem (or sometimes committed myeloid progenitor) cell, resulting in the proliferation of immature myeloid cells with a differentiation blockade. Only a subset of leukaemic cells are endowed with self-renewal capacity (so-called leukaemic stem cells, LSCs), a cellular hierarchy that can be fixed or plastic depending on the patient.

AML may occur *de novo*, or may result from the progression of an antecedent myeloid neoplasm, especially myelodysplasia, that is often not diagnosed before the AML phase (so called secondary-type AML) [1]. Clonal expansion may be favoured by exposure to chemotherapy or ionising radiation for the treatment of a previous, unrelated neoplasm [2].

The incidence of AML in adults is five to eight per hundred thousand per year in Europe; it increases with age, especially after the age of 50 [3]. An increasing number of studies have shown the important role of the tumour microenvironment within the haematopoietic niche on the development, progression and resistance to treatment of AML [4]. In this review, we will focus on one of the many players present in the bone marrow, endothelial cells (ECs). The role of the endothelium has been suspected and studied for more than 20 years in the process of leukaemogenesis [5,6], in resistance to chemotherapy and in the initial complications of AML [7].

The endothelium consists of a thin monocellular layer that covers the inner surface of all vessels of the body. ECs form an interface between the elements of the circulating blood and the vascular wall [8]. ECs are involved in multiple physiological functions, including the control of vasomotor tone [9–11], the maintenance of blood fluidity [12–15], the growth of new blood vessels [16–18] and cell trafficking. ECs show remarkable heterogeneity in terms of gene expression, antigen composition and function. Indeed, EC phenotypes vary between different organs and sometimes between different parts of the vascular loop within the same organ [19,20]. Endothelial heterogeneity may then explain the symptomatology observed in AML patients, with preferential organ involvement. Bone marrow ECs constitutively express E-selectin, Vascular Cell Adhesion Molecule (VCAM-1) and von Willebrand Factor [21,22]. They also express CD34, which is a ligand of L-Selectin implicated in the homing of haematopoietic stem cells (HSC) [23,24].

The recruitment of leukocytes into the tissues, also known as diapedesis, is a multistep process [25,26] involving the adhesion of leukocytes to the EC surface through adhesion molecules on the surface of the cells, the rolling of leukocytes on ECs [26–29], and the stimulation of leukocytes by chemokines secreted by ECs that activate leukocyte integrins, enabling firm adhesion and the arrest of leukocytes on the endothelial surface. Once firmly attached, leukocytes can cross the endothelium via a transcellular or paracellular pathway [25–27,30,31].

In this review, we will detail current knowledge of the role of the endothelium in AML, including endothelial involvement in the development, resistance and relapse of AML and its role in the different clinical manifestations of AML acute complications. Finally, we will discuss therapies that may target the interactions between leukaemic and endothelial cells.

2. Endothelial cells in acute myeloid leukaemia's microenvironment

HSCs are located in the bone marrow (BM) where they generate the cells needed to renew the blood and immune cell pool. This process requires strict regulation, with instructions provided by interactions with different actors within specific local microenvironments called niches. Multiple cells are present within the niche, including fibroblasts, osteoblasts/osteoclasts, adipocytes, neurons of the sympathetic nervous system, T lymphocytes, mesenchymal cells and ECs. All components of the BM microenvironment affect normal HSCs and leukaemic cells in various ways, impacting their cellular functions, including proliferation, differentiation, adhesion, quiescence, trafficking and clonal expansion [4,32–36]. HSCs are supposed to be distributed in 2 distinct locations: the vascular niche, located deep in the BM and rich in sinusoidal capillaries, and the endosteal niche, in close proximity to the inner surface of the bone. In both niches, HSCs specifically reside in perivascular areas [37]. Other works suggest that HSCs are mainly adjacent to sinusoidal blood vessels in the BM and spleen with a small percentage of HSCs localized near the endosteum[38,39].

Bone marrow ECs play a direct role in the support and regulation of HSC quiescence, self-renewal, activation, and homing [40]. In contrast to the majority of ECs in other vasculatures, they constitutively express P- and E-selectin, and loss of function studies have revealed that homing to the marrow niche is greatly impaired upon E- and/or P-selectin

deficiency [41,42]. Several selectin ligands are expressed on HSCs, including P Selectin Glycoprotein (PSGL)-1 and CD44, which regulates the rolling of HSCs [43–45]. The arrest of HSCs on the endothelium is established through interactions between Very Late Antigen (VLA)-4 and Lymphocyte Function-associated Antigen (LFA)-1 integrins, and their cognate receptors expressed on the microvasculature, VCAM-1 and Intercellular Adhesion Molecule (ICAM)-1, respectively [42,46,47].

Within the niche, interactions between HSCs and ECs may also increase the risk of clonal evolution of pre-leukaemic stem cells (pre-LSC) to leukaemic stem cells (LSC) [48–55]. Under inflammatory conditions, ECs produce Granulocyte Stimulating Factor (G-CSF) [56], potentially promoting the expansion of pre-malignant clones. The current main hypothesis is that leukaemia does not arise from a single LSC, but from several subclones in linear or branched architecture with different mutations, with each clone being supported by a pool of LSCs [57,58].

Glycoprotein CD44 has been reported as a marker of LSCs. CD44 binds to its major ligand hyaluronic acid, which is expressed by ECs. CD44 binding to hyaluronic acid increases VLA-4 expression and its affinity for its ligand VCAM-1 via the PI3k signalling pathway [59]. VLA-4, upon interaction with VCAM-1, promotes the survival and proliferation of AML cells through Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signalling, resulting in chemoresistance. The VLA-4-VCAM-1 interaction allows full integration of AML cells into the vascular niche and induces a quiescent phenotype in AML cells [60–62] (Figure 1). The inhibition of CD44 may also induce the differentiation of human AML cells with a need of further studies to confirm this effect [63,64].

Winckler *et al.* demonstrated that adhesion of HSCs to E-selectin promotes their proliferation, directly triggers their activation and induces lineage commitment. Conversely, the absence or therapeutic inhibition of E-selectin promotes HSC quiescence, HSC self-renewal potential and chemoresistance [65]. Barbier *et al.* highlighted the differences between leukaemic cells and HSCs. Indeed, in AML, E-selectin interactions favour malignant cell survival and chemoresistance *in vitro* and *in vivo* in a MLL-AF9 AML murine model. The genetic or therapeutic inhibition of E-selectin induces the cell cycling of leukaemic cells [66,67]. In this model, leukaemic cells secrete pro-inflammatory cytokines such as Tumour Necrosis Factor (TNF)- α that induce E-selectin surface expression on bone marrow ECs. Moreover, the binding of leukaemic cells to E-selectin leads to chemoresistance through the

establishment of efflux mechanisms into AML cells. It may also induce pro-survival signalling in AML cells via AKT/mTOR and NF- κ B pathways. Several adhesion molecules expressed by leukaemic cells bind E-Selectin, such as Cutaneous Lymphocyte Antigen-1 (CLA-1), PSGL-1, or CD44 [59,68–70].

Another key actor in AML cells-ECs interactions is the C-X-C Motif Chemokine Ligand 12 (CXCL12)/ C-X-C Motif Chemokine Receptor 4 (CXCR4) axis. CXCL12 binds to CXCR4, a G-protein-coupled chemokine receptor, which is functionally expressed in several cell types, including HSCs [71]. Similar to normal HSCs, the majority of AML blast cells also express CXCR4 [71–73]. The major sources of CXCL12 in AML BM are stromal cells and CXCL12-abundant reticular (CAR) cells, which are progenitors of mesenchymal stem cells [33]. ECs also secrete CXCL12 at lower levels [74]. The CXCL12/CXCR4 interaction activates several signalling pathways, including MEK/ERK, JAK/STAT, and PI3K/AKT axes, promoting cell survival [75]. Using an in-vitro model of AML cells culture, Schelker *et al.* have shown that CXCR4 is crucial for AML cell proliferation [76]. The ligation of CXCL12 to CXCR4 increases the activity of integrins such as VLA-4 and LFA-1[46] and subsequent adhesion of AML cells adhesion to bone marrow ECs [77]. Patients with higher expression of CXCR4 in AML cells have indeed decreased disease-free survival and lower response rates [78–80].

Finally, apoptosis or drug-induced leukaemic cell death leads to the release of leukaemic cytokines or extracellular histones that enhance the endothelial expression of adhesion molecules [7,81,82].

Collectively, these findings suggest that the cross-talk between leukaemic and endothelial cells could promote the adhesion of leukaemic cells to their niche, resulting in LSC quiescence, and chemoresistance [83–87].

Angiogenesis is increased in the BM of AML patients. Microvessel density is significantly higher in BM biopsies of AML patients at diagnosis compared with control patients. Microvessel counts were not related to age or sex of the patients in the AML group and in the control group [88,89]. After the induction of chemotherapy, microvessel density was significantly lower in patients achieving complete remission compared to those with persistent disease [88–90]. Immunohistochemical analysis of Vascular Endothelium Growth Factor (VEGF)-A protein in BM biopsies showed that AML patient BMs have a higher level of expression of VEGF-A compared with normal BMs. Moreover, higher circulating VEGF levels have an adverse impact on prognosis [91–95]. It has been suggested that AML cells activate

VEGF receptors through autocrine signalling to initiate self-renewal, proliferation, survival and resistance to chemotherapy. In parallel, paracrine VEGF signalling promotes angiogenesis.

Although highly vascularized, the hematopoietic niche is characterized by extremely low oxygen partial pressure. Hypoxia has been shown to be necessary for long-term maintenance of HSCs. Oxygen levels in BM range from 1% to 4%, decreasing from vessels to the endosteum [96,97]. Hypoxia-inducible factor (HIF) proteins are the main mediators involved in the adaptive response to hypoxia. Several genes activated by HIF have been involved in multiple cellular functions, including angiogenesis, energy metabolism, proliferation and survival [98,99]. In patients with AML, HIF-1 α expression is associated with a worse prognosis [100,101]. Severe hypoxia decreases leukemic cell-cycle activity, inducing a quiescent state that mediates resistance to chemotherapy [102].

Nitric oxide (NO) supports vascular homeostasis, including the regulation of vasodilative tone, the control of local cell growth and the defence of the vessel from the adverse effects of platelets and cells circulating in the blood, thus playing a crucial role in normal endothelial function [103]. Using intra-vital microscopy in a murine model of AML and patient-derived xenografts (PDX) of AML, Passaro *et al.* found increased vascular leakage compared to controls. Via transcriptomic analysis in the ECs of PDX and patient biopsies, they found that NO release by ECs was involved in capillary leakage. NO is also involved in the mobilisation of normal HSCs by decreasing their adhesion within the hematopoietic vascular niche. The inhibition of NO production in murine models of AML improves the response to treatment by reinstating the normal vascular niche, enhancing the drug response and maintaining residual bone marrow HSCs in the niche [104–106]. Through these signalling pathways, leukaemic cells can enhance vessel formation and maintain their own stem cells [107,108]. Cogle *et al.* demonstrated that leukaemic cells localise within the vascular endothelium in AML patients and in xenografted mice, where they adopt a quiescent phenotype [60]. ECs may therefore protect AML cells from cell cycle-targeting chemotherapies [60] (Figure 1).

3. Endothelial dysfunction is involved in AML-related organ failure

Five to 20% of patients with AML initially present with a high level of circulating leukocytes, exceeding 50-100 $\times 10^9/L$ [109–112]. These patients are exposed to life threatening complications with multiple organ failure, which may be aggravated by treatment. Hyperleukocytotic patients therefore have a higher risk of early mortality in the initial phase of the disease, varying between 6% and 20% [113–118]. Hyperleukocytosis is more frequently observed in monocytic AML (known as M4/M5 AML in the FAB classification) [119], in AML with MLL rearrangements [120] and in FLT3-mutated AML [121–123].

A Canadian study found an early excess mortality (death during induction chemotherapy) at a threshold of 30 $\times 10^9/L$ of leukocytes, with an increased risk of mortality correlated with WBC (white blood cell) counts, but without any impact of WBC count on complete remission, highlighting the fact that hyperleukocytosis is a risk factor for early death but not for response to chemotherapy. The main cause of death was bleeding [116]. In a French study of 189 newly diagnosed AML patients admitted to the ICU over a 10-year period, 43% had respiratory failure and most of them (61%) had lung infiltration of blast cells based on clinical and radiological examinations [118]. Patients with AML at diagnosis may also experience acute kidney injury (40% of the cases) requiring extra renal replacement therapy [124–126].

Beyond infections, hyperleukocytotic AMLs at diagnosis are complicated by leukostasis, solid organ infiltration, tumour lysis syndrome and disseminated intravascular coagulation (DIC) and we will develop the putative role of ECs in these different entities (Figure 2).

3.1 Leukostasis

Leukostasis consists of the aggregation of leukaemic cells circulating in the capillaries of different tissues such as the lung or central nervous system, and is responsible for alterations of gas exchanges with distension of the capillaries and tissue hypoxia by obliteration of the arterioles [127]. Increased blood viscosity appears to contribute to microcirculation abnormalities, with a correlation between the rate of circulating blasts and the risk of hyperviscosity [128]. Currently, the diagnosis of leukostasis is based on a clinical

presumption and the presence of circulating blasts [114]. However, additional factors may be involved, as indicated by the development of leukostasis in the absence of hyperleukocytosis or the failure of therapeutic trials with leukapheresis to control leukostasis [129,130].

Although there are few studies on the molecular mechanisms of leukostasis, the interaction between the blast and the EC seems to play an important role in this process.

Cell adhesion molecules such as L-selectin, E-selectin, VCAM-1, LFA-1 (CD11a/CD18) and integrin CD11b expressed on the surface of endothelial or leukaemic cells have been shown to be involved in the adhesion of leukaemic cells to TNF- α activated endothelium [7]. Leukaemic cells secrete several cytokines that will activate ECs and lead to the expression of adhesion molecules on their surfaces. On non-activated ECs, the presence of blasts induces the expression of ICAM-1, VCAM-1, and E-selectin. This activation is related to leukaemic cell secretion of TNF- α , Interleukin (IL)1- β , and direct contact between adhesion receptors expressed by blast cells and ECs. Thus, leukaemic cells have the ability to promote their own adhesion to the endothelium [7].

Patients with monocytic differentiated leukaemia (M4/5) have a higher risk of leukostasis [118,131]. The high level of CD11b expression on M4/M5 leukaemic cells (and its ability to bind ICAM-1 on ECs) may explain these results [7,132] (Figure 3).

3.2 Organ infiltration

Regardless of the level of circulating leukocytes, some patients appear to have blast-specific infiltration into solid organs such as the lungs, kidneys, skin or central nervous system [130,133,134]. Solid organ infiltration is also known as extramedullary AML and can now be evaluated by positron emission tomography/computed tomography (PET-CT) [135,136]. Although a disruption of the endothelium layer has been suspected to be crucial for leukemic extra-medullary infiltration, the mechanisms of organ failures are poorly studied. Stefanidakis *et al.* showed that co-culture of AML cells with ECs increases surface expression and colocalization of leukocyte integrins with pro-Matrix Metallo proteinase-9 (proMMP-9) on AML cells forming the "leukaemia cell invadosome". Using *in vitro* analysis, they demonstrated that the specific binding of MMP-9 to leukocyte surface β 2 integrin is required for pericellular proteolysis and migration of AML-derived cells. In a mouse model of

human AML xenograft, they found that blocking the binding of MMP9 to leukocyte integrins had a specific anti-leukemic activity[137].

Specific pulmonary infiltration is described in the literature as an extravascular pulmonary blast location. It is most often described in AML with partial or complete monocytic differentiation (AML4 and AML5) [131]. Specific pulmonary infiltration occupies the lymphatic vessels in the peribronchovascular spaces, the interlobular septum and the subpleural interstitial tissue [138]. In an autopsy study of patients with AML or non-hodgkin lymphoma who experienced acute respiratory failure, Van de Louw *et al.* found that more than 50% of patients had missed major clinical diagnoses. Among AML patients who presented with acute respiratory failure of unknown etiology, malignant lung infiltration was the main cause of death [139].

Renal infiltration has also been described in the literature with leukaemic cells within the interstitium and in the lumina of small vessels [140,141]. Enlarged kidneys may be observed in CT-scan or renal ultrasound but patients may also have only microscopic infiltration. Diffuse leukaemic infiltration may not systematically translate into acute kidney injury, as renal leukaemic infiltration has been reported as an incidental post-mortem finding [142]. However, in some cases, acute kidney injury results directly from AML cells infiltration of renal parenchyma [140]. Interactions between renal ECs and AML cells have never been specifically studied in this context of massive infiltration of leukaemic cells.

Leukaemia cutis is defined as the migration of leukaemic cells into the skin and has been described in AML patients with an estimated incidence from 10% to 50% in M4 and M5 subtypes [143–145]. The distribution of leukaemic cells in the skin is often perivascular, with a possible role for an interaction between adhesion molecules on the surface of blasts and adhesion molecules of ECs in dermal post-capillary venules [146].

Central Nervous System (CNS) involvement is less frequent in AML and occurs in 2–5% of patients [133,147–149]. The incidence of CNS leukaemia at relapse has decreased since the incorporation of high dose cytarabine in chemotherapeutic regimens. Indeed, until high-dose cytarabine was used, up to 20% of children and 16% of adults with AML had meningeal disease [150]. In order to infiltrate the CNS, leukaemic cells must cross the vascular endothelium of the blood brain barrier into the arachnoid, migrate and proliferate in cerebral spinal fluid and consequently infiltrate perivascular spaces and the brain

parenchyma. The role of the Neural Cell Adhesion Molecule (CD56) expressed by ECs is crucial for the entry of blasts through the blood-brain barrier [151–158].

3.3 Disseminated intravascular coagulopathy

AML associated coagulation disorders are also markers of endothelial dysfunction. Disseminated intravascular coagulopathy (DIC) is characterized by systemic intravascular activation of the coagulation system from various causes that can result in multiorgan failure, thrombosis, and/or excessive bleeding. The diagnosis of DIC is challenging due to the complex underlying medical conditions of leukaemia that can lead to variable presentations [159]. Indeed, diagnosis of DIC relies on decreased fibrinogen, increased prothrombin time and activated partial thromboplastin time, elevated D-Dimer, and thrombocytopenia. However, thrombocytopenia in these patients is rather related to BM infiltration than consumption of platelets. The incidence of DIC in AML, excluding acute promyelocytic leukaemia (APL), varies between 10% and 30%, with an increased risk (60%-70%) after the initiation of induction chemotherapy [160–163]. In non-APL AML, the main risk factor for developing DIC is an elevated WBC count [159,162,164]. This could be explained by Tissue Factor (TF) secretion by ECs secondary to the release of pro-inflammatory cytokines such as IL-1 from apoptotic leukaemic blasts [7,165]. Moreover, IL-1 blocks the anticoagulant protein C, shifting the balance on the endothelium from an anticoagulant state to a procoagulant state [165]. Chemotherapy-induced endothelial injury may also worsen the procoagulant state of patients with non-APL AML [166]. Indeed, anthracyclines may participate to endothelial damages [167–170]. Anthracyclines increase the production of mitochondrial reactive oxygen species (ROS), inducing ROS burst, leading to mitochondrial dysfunction and subsequent release of cytochrome c, activated caspases and apoptosis of ECs [171]. Moreover, in non-APL AML, the presence of microparticles that express TF has been shown to contribute to coagulation disorders [172–174]. Despite the bleeding risk associated with DIC, Libourel, EJ *et al.* have shown that DIC is also an independent risk factor for venous and arterial thrombosis [163].

In APL, coagulation disorders are present in more than 75% of patients at diagnosis. TF is present at the surface of leukaemic cells or at the surface of microparticles and is able to stimulate factor VII (FVII), which binds to FVIIa, thus activating FIX and FX. The latter leads to

thrombin generation, which itself catalyzes fibrinogen into fibrin. In parallel, Annexin II expressed at the surface of malignant leukocytes binds to tissue-Plasminogen Activator and plasminogen, resulting in the formation of plasmin, which cleaves fibrin and fibrinogen, in a process called fibrinolysis [175–179].

3.4 Chemotherapy-induced endothelial dysfunction

Chemotherapy drugs, and especially anthracyclines, induce endothelial damage [167–170]. Anthracyclines produce excess reactive oxygen species in the mitochondria, leading to mitochondrial dysfunction with the release of cytochrome c and the activation of caspases responsible for ECs apoptosis [171]. The other main drug used in AML is cytarabine-arabioside [180] which can induce capillary leak syndrome with the increased extravasation of fluid and protein through the endothelium, resulting in non-cardiogenic pulmonary oedema or serous effusions (pleura and pericardium) [181–186].

3.5 Tumour Lysis Syndrome induced endothelial dysfunction

Endothelial dysfunction may also contribute to tumour lysis syndrome (TLS) in AML. Indeed, recent data from our laboratory suggest that endothelial dysfunction has a major role in acute kidney injury during TLS. In a mouse model of AML driven by retroviral expression of a single mutational hit, the MLL-AF9 gene fusion, we found that endothelial dysfunction participates in TLS-induced acute kidney injury with a huge increase in plasma levels of extracellular histones that are released by dying cells. These histones induce profound renal endothelial dysfunction *in vitro* and *in vivo* [187].

4. The endothelium as a therapeutic target (Table 1)

Current treatment of non APL-AML relies mainly on an intensive chemotherapy regimen including an anthracycline (daunorubicin or idarubicin) combined with cytosine arabinoside. The treatment landscape of AML is evolving, with a dozen new drug approvals since 2017. These new effective treatments are directed against specific molecular pathways, discovered through advances in molecular biology, such as anti-FLT3 therapy[180]. Despite these advances, there is still a significant risk of relapse and death, requiring further clinical trials to improve the prognosis of patients. In addition, there are currently no randomised trials proving the efficacy of a treatment to reduce the risk of early morbi-mortality. In this part, we will focus on drugs targeting the interaction between AML cells and ECs.

4.1 Dexamethasone

Glucocorticoids and mainly dexamethasone can be used to treat leukostasis in patients with hyperleukocytic AML. Glucocorticoids are thought to have a demarginating effect on blasts adherent to the capillary walls, analogous to the effect of glucocorticoids on granulocytes, where the expression of adhesion molecules is reduced by glucocorticoids [188,189]. Glucocorticoids would therefore improve the flow of cells in the capillaries, whether pulmonary or cerebral, and improve clinical symptoms. In one retrospective study, dexamethasone efficiently treated leukostasis and lung specific infiltration [190]. Another team studied the effect of dexamethasone in both the induction phase and in the long-term relapse and survival of patients with hyperleukocytic AML. They found that dexamethasone combined with induction chemotherapy did not improve survival nor the complete remission rate. Interestingly, the authors found a significant reduction of the relapse rate in patients who were treated with dexamethasone in association with induction chemotherapy. Authors suggest multiple mechanisms of dexamethasone that may explain this result, including an inhibitory effect of dexamethasone on cytokine production by leukaemic cells and activated ECs that may decrease leukaemic cells chemoresistance. However, the link between dexamethasone, ECs and chemoresistance remains to be established [191].

4.2 Anti-angiogenesis

As described before, angiogenesis plays an important role in the development of AML. Therefore, therapies directed against VEGF and angiogenesis can help to improve the prognosis of patients. So far, various drugs with antiangiogenic activity have been evaluated in clinical trials, but the results have been disappointing [192]. Bevacizumab, a humanised anti-VEGF antibody, had a significant effect on the decrease in cellular VEGF expression in the bone marrow after 1–3 cycles, without displaying any significant anti-leukaemic activity in patients with relapsed/refractory AML [193]. Bevacizumab was also used in combination with induction intensive chemotherapy and showed promising results in a single arm phase I clinical trial in relapse/refractory AML patients with median overall survival of 16.7 months and median relapse free survival of 7 months. Serum VEGF levels and bone marrow microvessel density were significantly reduced [194]. However, a randomised phase II trial in elderly first-line patients in combination with chemotherapy did not show any efficacy in terms of overall survival or relapse-free survival [195]. There are no phase III studies to confirm these results. Tissue-specificity of ECs may also have participated in the failure of VEGF-targeted therapies [196]. Tyrosine kinase inhibitors like midostaurin and sorafenib target a broad spectrum of receptor tyrosine kinases, including VEGFR. They have been clinically investigated for the treatment of AML. Sunitinib had only modest clinical activity in the treatment of AML, while sorafenib, as a single agent or in combination with conventional chemotherapy, led to a reduction in leukaemic development only in AML patients with *FLT3* mutations [197–200]. Although tyrosine kinase inhibitors such as sorafenib have an *in vitro* effect on VEGFR, the fact that they only have a clinical activity in patients with *FLT3* mutated AML suggests that their effect on AML cells is rather due to *FLT3* inhibition than due to their anti-angiogenic activity.

Anti-angiogenic therapy, such as bevacizumab or tyrosine kinase inhibitor such as sunitib have also been shown to improve oxygenation and tissue perfusion in solid tumours [201–207]. In solid tumours, vascular damages such as capillary leakage and vessel compression may lead to focal hypoxia. As mentioned above, severe hypoxia participates to chemotherapy resistance in AML by decreasing leukemic cell-cycle activity. Anti-angiogenic therapies have shown promising results by correcting tissue hypoxia, inducing vascular

normalization and vessel decompression [208]. In AML, new antiangiogenic therapies have shown decreased proliferation and increased apoptosis of AML cells [209,210]. Further clinical studies focusing on anti-angiogenic therapies in AML are warranted.

4.3 Anti-adhesion therapies

As described above, there is mounting evidence that ECs play a key role in the development, relapse or resistance to chemotherapy of AML. Based on this evidence, teams are developing treatments that may break the link between leukaemic cells and ECs. These efforts resulted in the development of an E-selectin inhibitor, GMI-1271 or uproleselan [66]. In a mouse model of AML, Barbier *et al.* demonstrated that targeting E-Selectin inhibits the niche-mediated pro-survival signalling, impairs the recovery of AML blasts and acts synergistically with chemotherapy, doubling the survival time of mice compared to chemotherapy alone [66]. In a phase I/II clinical trial (ClinicalTrials.gov Identifier: NCT02306291), uproleselan was tested in 66 patients with AML in combination with chemotherapy. Overall, the 60-day mortality rate of all AML patients in the study was 9%, the median overall survival was 9.2 months (95% CI, 3-12.6) and event-free survival was 12.6 months (95% CI, 9.9-NA). Currently, uproleselan is being tested in AML patients in combination with chemotherapy and compared to chemotherapy alone in a randomised, double-blind, phase III trial in the USA, Australia and Europe (ClinicalTrials.gov identifier: NCT03616470)[211]. Another example of adhesion molecule targeted therapy is the development of anti-CD44 therapies. Anti-CD44 treatments induce the differentiation of AML blasts *in vitro* and increase sensitivity to chemotherapy [63,64,212,213]. Only one phase I dose escalation study has investigated anti-CD44 therapy using a recombinant anti-CD44 immunoglobulin G1 humanised monoclonal antibody as a single agent, in relapse/refractory AMLs. This trial enrolled 44 patients, 10 of whom received therapeutic doses of the drug. Pharmacokinetics increased supraproportionally, suggesting a target-mediated drug disposition at ≥ 1200 mg. Two patients achieved a complete response with partial response, and one patient had stable disease with haematological improvement. More clinical trials are needed to confirm the value of this treatment strategy [214].

Drugs targeting CXCL2/CXCR4 axis, such as plerixafor, a CXCR4 antagonist, or ulocuplumab, an anti-CXCR4 monoclonal antibody are also under current evaluation for the treatment of

AML. *In vitro* study showed that inhibition of CXCL2/CXCR4 pathway restored AML cells sensitivity to chemotherapy [75,76,215–218]. Anti-CXCL2/CXCR4 therapies have also been used in murine model of AML and patient-derived xenografts and have demonstrated significant reduction of tumor burden[75,219,220]. Several phase I/II study in humans with plerixafor and other targeted therapy against CXCR4 found that it is a feasible treatment with significant anti leukemic activity but larger phase III studies are needed to confirm the clinical benefit for patients[221–224].

5. Conclusion and future directions

Emerging data suggest a crucial role for ECs in the pathophysiology of AML. By binding to ECs through different adhesion molecules, leukaemic cells can invade different organs, protect themselves from chemotherapy, and then participate in the relapse of disease. The use of drugs directed against these adhesion molecules appears to be an interesting strategy to treat patients with AML and to prevent specific complications. Future work should focus on the role of ECs according to their tissue of origin by separating ECs from the bone marrow, lung or kidney and according to the vessel, whether it is a capillary, a venule or an arteriole. The treatment of AML requires the clearance of LSCs, which may be achieved by combining standard chemotherapy with drugs targeting the binding between ECs and leukaemic cells; these should therefore be developed and evaluated in therapeutic trials. Finally, understanding the pathophysiology of acute complications in patients with newly diagnosed AML may lead to new therapeutic responses, probably focusing on the use of anti-adhesion drugs.

6. Practice points

- Through adhesion molecules, endothelial cells (ECs) bind to leukaemic cells in the bone marrow niche, protecting leukaemic cells from death
- Leukaemic cells enhance their ability to bind to ECs, by secreting cytokines that increase the level of adhesion molecules on the surface of ECs
- By secreting VEGF, leukaemic cells increase the density of capillaries within the niche, further modifying their microenvironment to their advantage
- Leukaemic cells have the ability to bind to ECs of the pulmonary and cerebral capillaries via adhesion molecules resulting in a leukostasis syndrome
- Leukaemia cells activate endothelial cells leading to activation of coagulation and eventually to DIC

7. Research agenda

- Clinical outcome of anti-adhesion drugs on remission, overall survival and relapse-free survival
- Decipher *in vitro* and *in vivo* interaction between bone marrow ECs and leukaemic cells to identify new therapeutic targets
- Decipher *in vitro* and *in vivo* mechanism of leukostasis to identify therapeutics target

Figure legends

Figure 1. EC and leukaemic cell interactions within bone marrow niches

Leukaemic cells may promote their survival by remodelling endothelial cells (ECs) in the bone marrow. Leukaemic cells secrete Vascular Endothelium Growth Factor-A (VEGF-A), which increases angiogenesis and, via an autocrine signal, could enhance leukaemic cell renewal and resistance to chemotherapy. The cross-talk between leukaemic cells and ECs increases the adhesion of blast cells to ECs. Leukaemic cells secrete pro-inflammatory cytokines that activate ECs and increase the expression of adhesion molecules on their surface (E-selectin, VCAM-1). Once bound to the ECs, leukaemic cells also increase the expression of VLA-4 on their surface via Pi3 kinase signalling, thus reinforcing the link between the leukaemic cells and the ECs. The subsequent activation of intracellular signalling pathways involving AKT/mTOR and NF- κ B pathways in leukaemic cells induces a decrease of chemotherapy-induced apoptosis. Nitric Oxide (NO) release by activated ECs is involved in capillary leakage. Severe hypoxia in the bone marrow through Hypoxia Induce Factor (HIF) pathway decreases leukemic cell-cycle activity, inducing a quiescent state that mediates resistance to chemotherapy and stimulate angiogenesis.

Figure 2. Specific acute complications of AML

Patients with acute myeloid leukaemia, especially patients with hyperleukocytosis $>50G/L$, are at risk of organ failure due to leukaemia-specific complications. Leukostasis consists of an aggregation of leukaemic cells circulating in the capillaries of different tissues and is responsible for alteration of gas exchanges with distension of the capillaries and tissue hypoxia by obliteration of the arterioles. Leukostasis can be responsible of respiratory or neurologic failure. Solid organ infiltration is described in the literature as an extravascular leukaemic cell location and can affect almost every organ. The tumor lysis syndrome, often secondary to the initiation of a specific treatment, but which may also be spontaneous, consists of the death of cancer cells which release toxic products (potassium, uric acid, etc.) into the circulation, as well as extra-cellular histones capable of activating the endothelium and causing microvascular lesions in different organ, mainly the kidney but also the lung or the nervous central system.

Figure 3. Endothelium involvement in acute complications of acute myeloid leukaemia

Leukaemic cells promote their adhesion to endothelial cells (ECs) via the secretion of soluble factors such as the cytokines TNF- α or IL-1 β or extra-cellular histones. These induce the ECs activation and expression on surface of adhesion molecules, such as Intercellular Adhesion Molecule 1 (ICAM-1), Vascular Cell Adhesion Molecule 1 (VCAM-1) and E-selectin. Leukaemic cells bind to these adhesion molecules via their ligands such as Very Late Antigen-4 (VLA-4), P-Selectin Glycoprotein Ligand-1 (PSGL-1) or Lymphocyte function-associated antigen 1 (LFA-1), aggravating leukostasis in the capillaries. Leukaemic cells attached to the microvessel wall also secrete Matrix Metalloproteinases (MMPs), favouring solid organ infiltration by leukaemic cells. Disseminated Intravascular Coagulation (DIC) may be a consequence of increased Tissue Factor (TF) expression by microparticles (MPs) released by leukaemic cells, and of increased TF expression by activated ECs.

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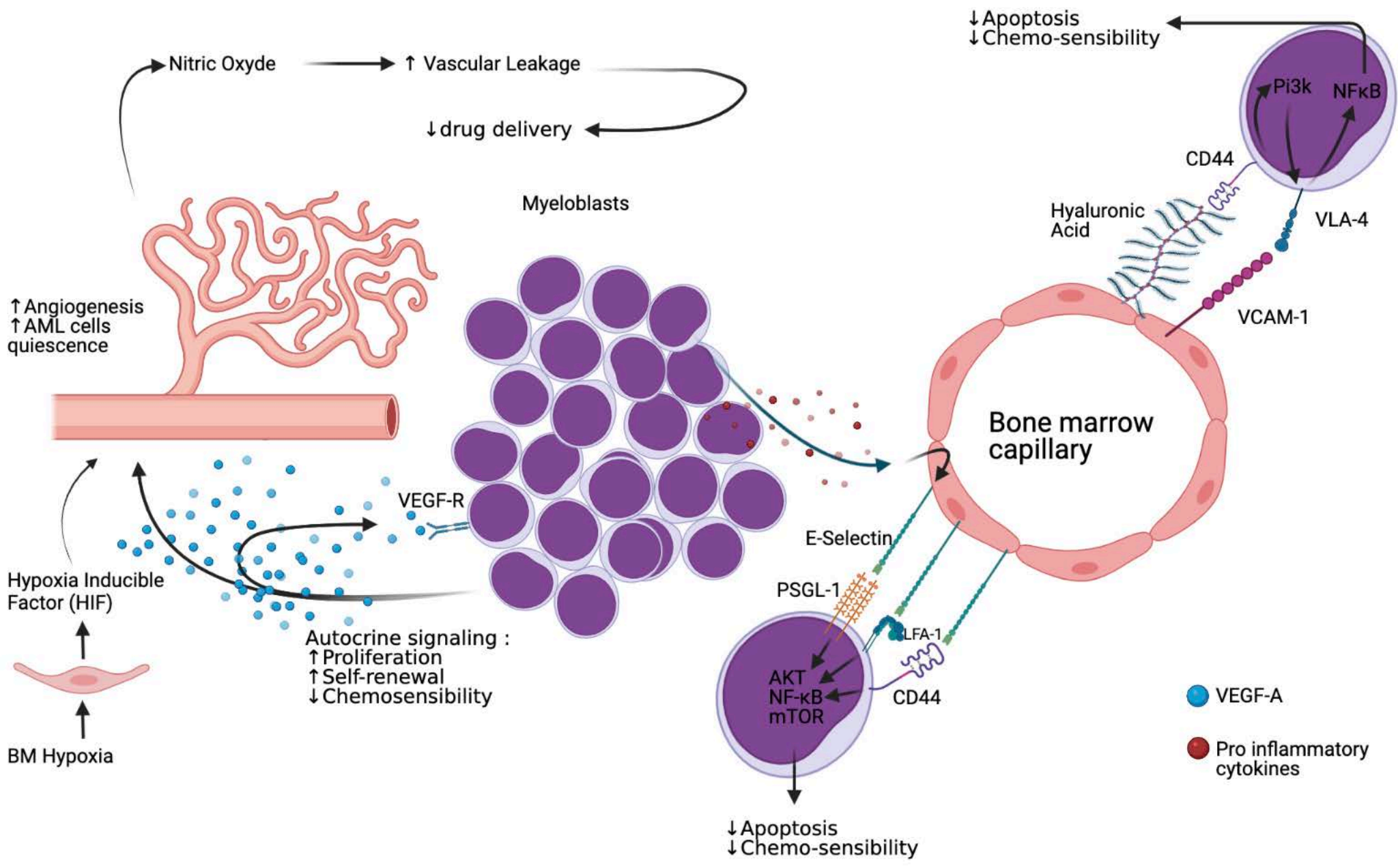
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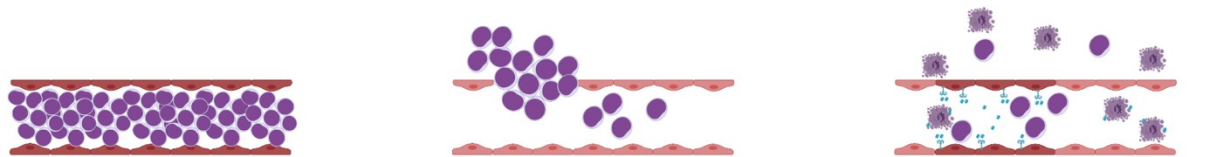
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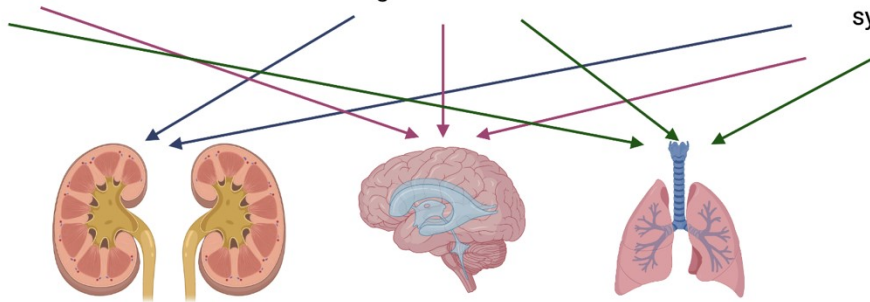









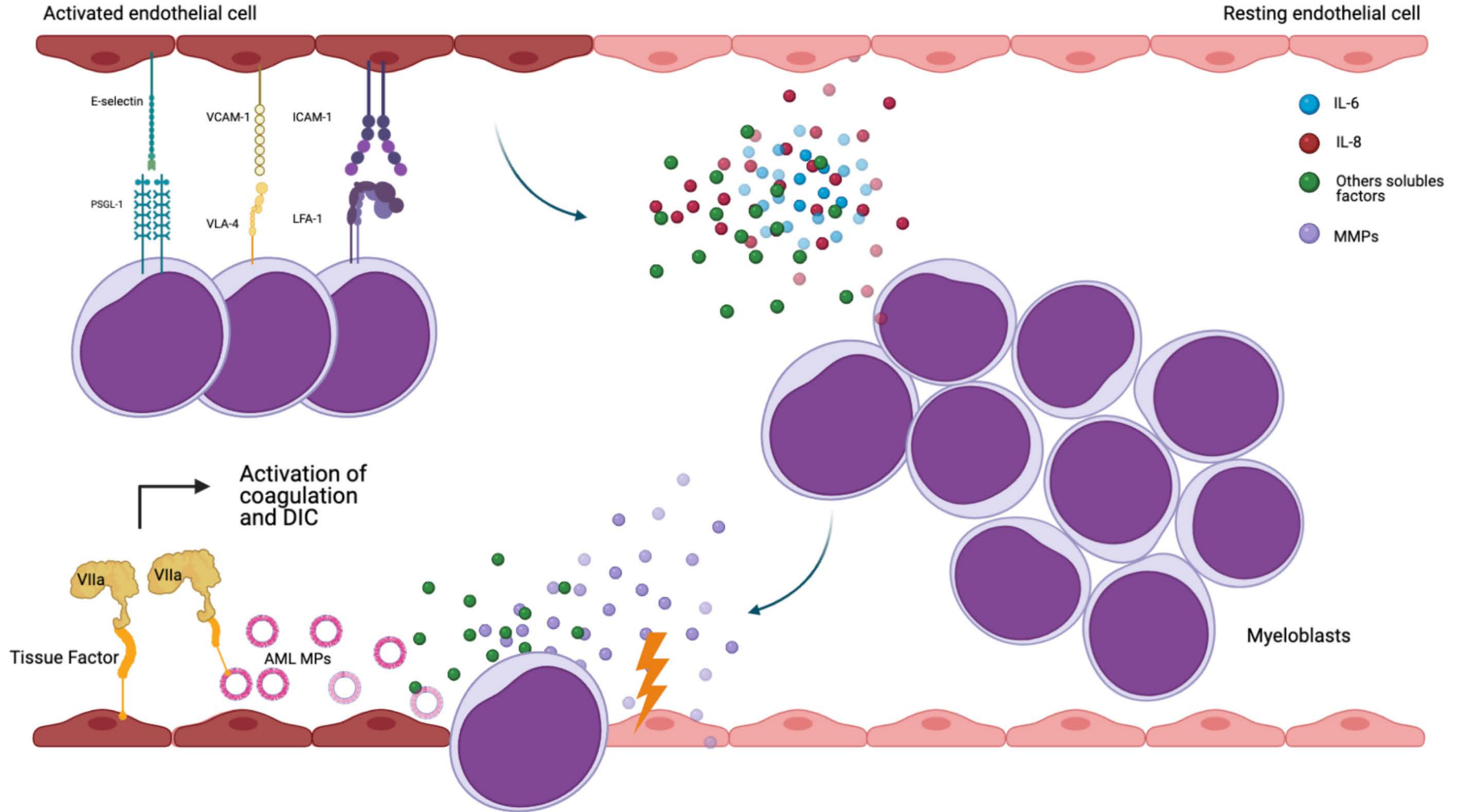
Leukostasis

Organ infiltration

Tumor lysis syndrome



-  Resting endothelial cell
-  Myeloblast
-  Activated endothelial cell
-  Dying myeloblast
-  Extra-cellular Histone



Therapeutic target	Drug	Pharmacodynamic	Desease status	Study design	On going/Finished	References
∅	Dexamethasone	sensitizing leukaemic cell to chemotherapy	Relapsed/Refractory AML	Phase 3	On going	[225] NCT03765541
VEGF	Bevacizumab	recombinant humanized IgG, anti-VEGFR	Newly Diagnosed AML	Randomized Phase 2	finished	[195]
VEGF	Bevacizumab	recombinant humanized IgG, anti-VEGFR	Relapsed/refractory AML	Phase 1	finished	[226]
VEGF-R	Sorafenib	Tyrosine Kinase Inhibitor, anti-VEGFR activity	Newly diagnosed AML	Phase 1/2	On going	[227] NCT02728050
E-Selectin	Uproleselan (GMI-1271)	E-Selectin antagonist	Newly diagnosed AML	Phase 2/3	On going	[228] NCT03701308
E-Selectin	Uproleselan (GMI-1271)	E-Selectin antagonist	Relapsed/refractory AML	Phase 3	On going	[229] NCT03616470
CD44	MLM-CAR44.1 T-cells	CAR-T Cell targeting CD44	Relapsed/refractory AML	Phase 1/2	On going	[230] NCT04097301
CXCR4	Plerixafor	CXCR4 antagonist	AML in CR before alloHSCT transplantation	Phase 2	Terminated, results unpublished yet	[231] NCT02605460

Table 1. Potential therapeutics to target leukaemic cell-ECs interaction