REVIEW ARTICLE

The angioregulatory cytokine network in human acute myeloid leukemia – from leukemogenesis via remission induction to stem cell transplantation

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ABSTRACT. Acute myeloid leukemia (AML) is characterized by bone marrow accumulation of immature leukemic blast cells. Conventional AML treatment includes induction chemotherapy to achieve disease control, followed by consolidation therapy with conventional chemotherapy or allogeneic/autologous stem cell transplantation (allo/auto-SCT) to eradicate residual disease. Even younger patients receiving the most intensive treatment have a median, long-term, AML-free survival of only 45-50%, highlighting the need for new treatment strategies. The important role of the bone marrow cytokine network during disease development and treatment is suggested by several observations, including: (i) the increased microvascular density (MVD) in leukemic bone marrow, (ii) experimental evidence of cytokine-mediated crosstalk between leukemic and microvascular endothelial cells, (iii) the prognostic impact of angioregulatory cytokine levels both in patients receiving conventional chemotherapy and allo-SCT, and (iv) the experimental evidence for an antileukemic effect of cytokine inhibition in human AML. Several cytokines are constitutively released by human AML cells, including interleukins, chemokines, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and angiopoietins. However, the cytokine system constitutes a functional, interacting network, and recent evidence suggests that analysis of serum cytokine profiles rather than the analysis of single cytokines should be used for prognostic evaluation of AML patients. We will discuss the role of angioregulatory cytokines in leukemogenesis, including their direct effects on the leukemic cells, as well as their indirect contribution to leukemogenesis through angioregulation and crosstalk between leukemic and neighboring stromal cells. We shall also discuss the possibility of targeting angioregulatory cytokines as a part of the treatment strategy in leukemia.

Key words: acute myeloid leukemia, cytokines, bone marrow, angiogenesis, stem cell transplantation

Acute myeloid leukemia (AML) is the most common myeloid malignancy, the median age at diagnosis being 65-70 years [1]. The disease is heterogeneous with regard to clinical and biological characteristics, although all patients show a similar bone marrow accumulation of immature leukemia blasts [1]. AML is usually an aggressive malignancy; the median survival is only 2-4 months for patients only receiving supportive therapy [2], and the disease-free survival rate is only 40-50%, even for the younger patients (<60-65 years of age) who can receive the most intensive, antileukemic treatment [1]. However, the prognosis after intensive therapy also differs between patients [1], and analysis of cytogenetics and molecular genetics is used to evaluate the relapse risk and decide the optimal treatment for individual patient [1, 3-5]. Furthermore, AML bone marrow is an interacting network of leukemic cells and nonleukemic stromal cells. Bone marrow microvessel density is increased in human AML, and the local cytokine network is probably important for this disease-associated angiogenesis [6]. In the present article, we review (i) how angioregulatory cytokines contribute to leukemogenesis, (ii) the modulation of the angioregulatory cytokine network during antileukemic treatment, and (iii) the possible targeting of angioregulatory cytokines in AML therapy.

CLINICAL BACKGROUND – CURRENT CHALLENGES IN AML THERAPY

The primary objective for the chemotherapy of AML is to induce complete hematological remission (*i.e.* disease control) through the initial induction treatment and thereafter to reduce the risk of relapse from residual disease through consolidation therapy [7]. The induction treatment is often an anthracycline combined with cytarabine [7, 8]; both drugs cause DNA damage, thereby triggering programmed cell death. Attempts to improve the induction treatment using other anthracyclines [7, 9], high-

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dose cytarabine [8], combination with additional cytotoxic agents [9], or priming of the AML cells through the administration of hematopoietic growth factor granulocyte colony-stimulating factor (G-CSF) [10] or granulocyte-macrophage colony-stimulating factor (GM-CSF) [11] have generally failed even though the growth factor treatment may have an effect in certain patient subsets [10, 11]. The consolidation therapy can be conventional, intensive chemotherapy [12] or high-dose chemotherapy followed by autologous or allogeneic stem cell transplantation (auto-SCT or allo-SCT) [13, 14]. Allo-SCT is the most powerful treatment, with antileukemic effects mediated both by the pre-transplant, high-dose conditioning therapy and by immune-mediated graft-versus-leukemia (GVL) effects [7, 13, 15-17]. Allotransplantation is associated with the high risk of early, transplant-related mortality, and reduced-intensity conditioning (RIC) is now tried to reduce this risk [18].

The most important improvements in the treatment of AML over the last two decades have been effected by the optimal use of well-known DNA-damaging drugs and improved supportive care [17]. Specific therapeutic targeting of intracellular signaling is now considered in AML treatment, but the results from initial clinical studies of gemtuzumab ozogamacin [19], FLT3 inhibitors [20], farnesyltransferase inhibition [21], histone deactylase (HDAC)-inhibitors [22] and bortezomib [23] have been disappointing. This is also true for the majority of elderly patients >60-70 years of age who often have more aggressive disease and cannot receive the most intensive treatments [24, 25].

LEUKEMOGENESIS – THE CONTRIBUTION OF ENDOTHELIAL CELLS AND ANGIOREGULATORY CYTOKINES TO AML DEVELOPMENT

Evidence for angiogenesis as a part of leukemogenesis in human AML

Angiogenesis is regulated by the balance between pro- and antiangiogenic cytokines [26]. Cancer-associated angiogenesis was originally described in solid tumors [27], but recent studies suggest that angiogenesis is also important for disease development and chemosensitivity in diffusely infiltrating bone marrow malignancies. Firstly, bone marrow MVD is often increased in hematological malignancies, especially in patients with advanced stage disease [28]. Secondly, antiangiogenic treatment causes vascular disruption and has antileukemic effects [29]. Thirdly, the cytokine crosstalk between leukemic and microvascular endothelial cells can increase the proliferation of both the endothelial [30] and leukemic cells [31]. Finally, the use of magnetic resonance imaging of the bone marrow vascularity may be used in the prognostic evaluation of AML patients [32].

Expression of cytokines by bone marrow endothelial cells

Endothelial cells show constitutive or inducible expression of various chemokines and adhesion molecules; different expression patterns are seen in various vascular beds and this seems to depend on both signals from the local microenvironment and on epigenetic differences [33]. The bone marrow contains small blood vessels (*i.e.* the sinusoids) that have unique structural and functional properties [34]. Primary bone marrow endothelial cells (BMECs) express adhesion molecules such as E-selectin and CD31 (PECAM) [34, 35], which have been reported to play a role in the homing of hematopoietic progenitor cells [36-38]. In addition, BMECs express several cytokines, cytokine receptors and adhesion molecules that promote hematopoiesis and are also involved in stem cell mobilization [35, 39, 40]. Some of the molecules expressed by primary BMECs and cell lines are shown in *table 1*, and in particular CXCL12 and stem cell factor (SCF) are important for the maintenance of hematopoietic stem cells (HSC) in bone marrow niches (see below).

The vascular stem cell niche

Bone marrow niches are specialized microenvironments involved in the maintenance of hematopoietic stem cells (HSCs). Most studies of HSC niches have focused on the endosteum because osteoblasts and osteoclasts seem to have a regulatory role, either directly or indirectly in HSC self-renewal [41]. More recent studies of HSCs have shown that areas adjacent to sinusoids may constitute an HSC niche, which has been termed the vascular niche (or perivascular niche). Firstly, a large proportion (about 60%) of purified HSCs, identified by the signaling lymphocyte activation molecules (SLAM) family markers, were found to reside in close association with sinusoidal endothelium [42]. Secondly, endothelial cells express several molecules that play a role in hematopoiesis [43], and several factors released by endothelial cells may contribute to HSC maintenance. Studies by Butler *et al.* [44, 45] have suggested that the ability of endothelial cells to support HSC expansion and self-renewal is orchestrated by angiocrine factors, including Notch ligands and stem cell factor (SCF). A recent study also implicated a role for endothelial cells in HSC maintenance through SCF production [46], and HSC activity seems to be increased in mice after activation of AKT1 in endothelial cells [47]. Thirdly, endothelial cells may constitute a site of differentiation for hematopoietic cells. A study by Avecilla *et al.* [48], showed that BMECs promote megakaryocyte maturation and platelet production through chemokine-mediated signaling involving CXCL12 and FGF-4. Finally, endothelial cells have been shown to play an important role in HSC engraftment after SCT, which seems to depend on VEGFR2-signaling [49]. Another study also implied that VEGF-induced upregulation of Tie-2 expression was involved in regeneration of vasculature and hematopoietic recovery after bone marrow suppression [50]. Thus, these studies suggest an important role for endothelial cells in HSC maintenance and hematopoietic regeneration *in vivo* through secretion of soluble factors.

Bone marrow niches regulate HSC self-renewal, proliferation, differentiation and quiescence, although the exact function of the vascular niche is currently under debate. Endothelial cells can promote HSC maintenance, however as HSC traffic in and out of the vasculature, it has been suggested that sinusoidal endothelium functions as a temporary place of residence where HSC proliferate and differentiate before entering circulation. Sinusoidal blood vessels are found in the central region of the marrow, but

Table 1
Expression of various molecules by human BMEC.

Molecule expressed	Endothelial cell investigated	References
Growth factors and cytokines		
G-CSF, GM-CSF, SCF, FGF, IL11, IL6, IL1-α, TGF-β, Nitric oxide	Primary cells and cell line Cell line	[39, 43, 146, 147]
IL5 after stimulation	Primary cells	[148]
Leukemia inhibitory factor (LIF)	Primary cells	[149]
Chemokines		
CXCL12, CXCL10*, CXCL8, CCL5*, CCL2	Cell line	[150]
CXCL8, CCL3	Cell line	[147]
Chemokines receptors		
CCR3-4, CXCR1-5	Cell line	[150]
Adhesion molecules		
CD62e (E-selectin)*, CD106 (VCAM-1)*, CD54 (ICAM-1), CD31 (PECAM-1), CD29 (fibronectin receptor), CD49b/c/d	Primary cells and cell line	[146, 151]

* Inducible by inflammatory cytokines.

are also found near to the endosteum [51], and both these areas are in close contact with other cell types including CXCL12-abundant reticular (CAR) cells [52], which express high levels of CXCL12, and these cells are thus suggested to be key components of HSC niches. Taken together, sinusoidal endothelium seems to be involved in HSC maintenance, and regulation of hematopoiesis. However, it is still not known which cell types have the major responsibility for creation of the niche, if there is cross-talk between multiple niches, or if the functions of the niches are unique [53].

Myoepithelial pericytes – a possible participant in bone marrow angiogenesis?

Pericytes are important for small vessel stabilization; they constitute a capillary-stabilizing network, and can be identified by their expression of α-actin, desmin, and the receptor for PDGF-B [54]. These cells also seem to be important for the control of endothelial cell differentiation and proliferation [54]. Pericytes are also present in normal human bone marrow, where they coat the microvssels [55, 56]. The possible role of pericytes in AML bone marrow has not been investigated, but cytokine-mediated crosstalk in an interacting triangle of pericytes, endothelial cells and leukemic blasts, may also be important for leukemogenesis in human AML [57]. CXCL12 is important both in AML development and pericyte differentiation, and even though this chemokine is not released by AML cells [58-60] it is present in the bone marrow and may thereby induce pericyte differentiation, together with the PDGF-AB release by primary human AML cells [61].

Constitutive release of angioregulatory cytokines by primary human AML cells

Primary human AML cells show constitutive release of a wide range of angioregulatory cytokines, including vascular endothelial growth factor (VEGF) [6, 62-66], hepatocyte growth factor (HGF) [67-72], several CCL and CXCL chemokines [58, 59, 73-75], Angiopoietin (Ang)-1 and Ang-2 [66, 69, 76-80]. The biological characteristics and their possible importance in leukemogenesis and chemosensitivity in human AML are summarized in *table 2*. The levels of several angioregulatory cytokines are associated with prognosis for patients receiving intensive chemotherapy, an observation suggesting that they are involved in the regulation of apoptosis and are important for chemosensitivity. Primary human AML cells also show constitutive release of several matrix metalloproteases (MMPs) and tissue inhibitors of MMPs (TIMPs) that are involved angiogenesis through their role in matrix degradation. However, these mediators also have additional effects and interact with the chemokine system through their protease activity with cleavage and activation [81, 82]. Some of the TIMPs also seem to act as signaling molecules, independent of their protease inhibitory effect, but it is not known whether these effects are important in human AML. Finally, as summarized in *table 2*, specific inhibitors of the proangiogenic cytokines have been developed; many of these are now in early clinical trials and CXCL4 inhibition is even used in routine clinical practice as a stem cell mobilizer [83].

LEUKEMOGENESIS AND CHEMOSENSITIVITY – THE SYSTEMIC CYTOKINE PROFILE IN PATIENTS WITH UNTREATED AML

Primary human AML cells show constitutive release of a wide range of cytokines and chemokines, and increased systemic (plasma/serum) levels of single cytokines are associated with adverse prognosis, *i.e.* VEGF, HGF and Ang-2 [64, 78, 79, 84-86] (*table 2*). However, cytokine action is contextual and a part of the cytokine networks [87]. It therefore seems rational to study cytokine networks in addition to single cytokine levels. This has recently been performed in untreated AML by Kornblau *et al.* [88]. They examined 290 patients, most of them below 60 years of age who they received later intensive treatment than usual. Distinct, systemic cytokine- and chemokine signatures were found, these were altered compared to healthy controls and had prognostic impact; they were also associated with remission rate (*i.e.* primary resistance), risk of later relapse and overall survival. We have performed a similar study

Table 2
Angioregulatory cytokines constitutively released by primary human AML cells; biological and clinical relevance for human AML.

Cytokine [REFS]	Cytokine and cytokine receptors	Biological effects	Role in AML	Pharmacological targeting
VEGF [6, 62-66]	**The VEGF family:** VEGFA, VEGFB, VEGFC, VEGFD and placental growth factor **Receptors:** two related RTKs of VEGFR1 and VEGFR2	**Endothelial cells**: support growth, migration and survival of endothelial cells mainly through ligation of VEGFR2 **Vessels:** critical for blood vessel formation and regulation of vascular permeability	**Release:** Released by bone marrow stromal cells, but also constitutive release by the AML cells for a subset of patients **Prognosis:** high intracellular levels and serum levels are associated with adverse prognosis	**Neutralizing antibodies** to VEGF or VEGFRs; **Soluble VEGFRs** or chimeric receptors that trap circulating VEGF **VEGFR TKIs**.
HGF (MET ligand) [67-72]	**Receptor:** MET; HGF is the only ligand **Signaling:** HGF ligation activates the RTK activity and initiates intracellular signaling; the most important being PI3K and STAT pathways	**Endothelial cells:** supports growth, migration, survival differentiation	**Release:** bone marrow stromal cells, constitutive release by AML cells for a subset of patients **Prognosis:** high serum levels are associated with adverse prognosis	**Biological HGF/MET antagonists** include truncated or uncleavable HGF forms that antagonize full-length HGF **Neutralizing monoclonal antibodies** directed against HGF or MET **Synthetic MET kinase inhibitors** that antagonize intracellular ATP binding
CCL chemokines [58, 59, 73, 74]	CCL1 **Receptor:** CCR8 CCL2 **Receptor:** CCR2 CCL3 **Receptor:** CCR5	**Angiogenic chemokines:** (i) recruitment of angiogenic hematopoietic cells; (ii) activation of endothelial cell chemotaxis and tubular formation; (iii) stimulating angiogenic growth factor signaling; (iv) direct stimulating and activation of RTKs	**Constitutive release** by primary AML patients for most patients	**Broad-spectrum chemokine inhibitors** (*e.g.* NR58-3.14.3) and more **specific inhibitors** are under developing
CXCL8	**Receptor:** CXCR1,2	**Angiogenic chemokines:** (i) recruitment of angiogenic hematopoietic cells; (ii) activation of endothelial cell chemotaxis and tubular formation; (iii) stimulating angiogenic growth factor signaling; (iv) direct stimulating and activation of RTKs	**Constitutive release** by primary AML patients for most patients	
CXCL9-11	CXCL4 **Receptor:** CXCR3B CXCL9-11 **Receptor:** CXCR3B	**Angiostatic chemokines:** (i) recruitment of T-cells which induce expression of angiostatic factors; (ii) activation of endothelial cell apoptosis and regression Inhibition of angiogenic growth factors; (iii) direct binding and inhibition of RTKs	**Constitutive release** by primary AML patients for most patients	
Angiopoietin 1/2 [66, 69, 76-80]	**The Ang family:** Ang 1-4, Ang1 and Ang2 are most relevant for angiogenesis. Ang1 is a Tie2 agonist, Ang2 is context-dependent Tie2 partial agonist/antagonist. **Receptor:** Tie1 and Tie2 are RTK receptors, Tie 2 is most relevant in angiogenesis	**Tie2 ligation by Ang1** strengthens the interaction between endothelial and periendothelial cells, Ang2 disrupt these interactions. **Ang1:** Released by non-endothelial cells and binding Tie1 and Tie2 **Ang2:** Released by endothelial cells and binding Tie 1	**The prognostic impact of Ang2 is controversial:** High protein levels in bone marrow are associated with good prognosis, high mRNA expression in bone marrow and high plasma levels have an adverse prognosis. High plasma levels are associated with unfavorable outcome after allo-SCT **Ang1 release:** Low-risk NPM1 mutations are associated with high constitutive Ang1 release by primary AML cells. Ang1 release is further increased in the presence of endothelial cells **Constitutive Ang2 release** is seen for a minority of patients	**Selective Ang1/Ang2 or Tie2 antibodies:** Decrease angiogenesis and inhibits tumor growth in experimental models

Table 2
(*Continued*)

Cytokine [REFS]	Cytokine and cytokine receptors	Biological effects	Role in AML	Pharmacological targeting
MMPs [81, 82]	**MMP-2, MMP-7, MMP-9, MMP-10:** Quantitative most important in AML	Complex functions involved in proliferation, antiapoptose, angiogenesis, invasiveness and cytokine processing	**MMPs** are both membrane bound and secreted by AML cells, secreted by stromal cells (MMP-2)	**MMP inhibitors (MMPIs):** Decrease angiogenesis and inhibits tumor growth *in vitro*. Limited *in vivo* results
TIMPs [81, 82]	**TIMP-1, TIMP-2:** Quantitative most important in AML	Inhibit MMP function, also involved in apoptosis and proliferation	**TIMP-1 and TIMP-2** are secreted by majority of AML patient cells	**Selective TIMP antibodies**

Abbreviations: ATP, adenosine triphosphate; RTK, receptor tyrosine kinase; TKI, tyrosine kinase inhibitor; MET, Mesenchymal-epithelial transition factor; VEGF, VEGF receptor

involving 82 unselected and untreated AML patients, but we also included patients with AML relapse. Our patients also had a higher median age and included 42 patients above the age of 60 years who only received disease-stabilizing treatment owing to their age and/or comorbidity [89]. We could not investigate the possible prognostic impact of the pretherapy cytokine profiles because of the heterogeneity with regard to treatment regimen. Both our study and the study by Kornblau *et al.* compared pretherapy AML serum profiles with healthy controls; the results from these two AML studies are summarized in *table 3*. The two studies had 23 overlapping cytokines; 14 of these cytokines showed similar alterations in the two studies, whereas differences were observed for nine mediators. One possible explanation for this discrepancy between the two studies could be the greater age of our patients; age-dependent differences in serum cytokine levels in AML have been described previously [84]. However, it should be emphasized that the differences were seen mainly for interleukins (IL2, IL4, IL5, IL10, IL12) that showed similar low levels in both studies, however, these differences were relatively small. Serum cytokine profiles have also been investigated in patients with preleukemic myelodysplastic syndrome (MDS) and aplastic anemia [88, 90]. The cytokine profiles in AML patients seem to differ from the profiles both in MDS and aplastic anemia patients, but the overall cytokine profile may have a diagnostic value in the differentiation between aplastic anemia and hypoplastic MDS [90].

THE CYTOKINE PROFILE FOR ACUTE LEUKEMIA PATIENTS AFTER REMISSION INDUCTION – PRETRANSPLANT CYTOKINE PROFILES VERSUS RISK OF EARLY COMPLICATIONS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

The antileukemic effect of allo-SCT is due to the ablative conditioning therapy, nonspecific graft-versus-host (GVH) reactivity and specific GVL reactivity [91], but there is still a risk of AML relapse [92]. Allotransplantation is, in addition, associated with a relatively high non-relapse or treatment-related mortality due to infection, severe graft-versus-host disease (GVHD), and chemotherapy-induced organ toxicity [13]. Cytokines are then important both for the relapse risk, hematopoietic and immune reconstitution, and development of post-transplant complications [93]. This is reflected in the significant correlations between pretransplant serum levels of single soluble mediators and prognosis, *i.e.* high pretransplant levels of angioregulatory Ang-2, as well as its receptor Tie2 and adverse prognosis after allo-SCT [79, 94]. In one of these studies, study serum Ang-2 levels were determined for 90 patients with AML or high risk MDS before allo-SCT [79]; the Ang-2 levels were then a predictor for time-to-relapse, however, there was no significant association with overall survival [79]. Increased pretransplant serum Tie2 levels seems to reflect residual leukemic disease burden [94] and was an independent predictor both for post-transplant AML relapse and overall survival [94].

G-CSF-mobilized allogeneic peripheral blood stem cell (PBMC) grafts contain an increased number of mature T cells, but despite this, the incidence of T cell-dependent, severe GVHD (especially acute GVHD) is not significantly increased compared with bone marrow transplantation (BMT) [95, 96]. An immunosuppressive effect of G-CSF on donor T cells in the grafts probably contributes to a protective effect of G-CSF against GVHD, including both the direct effects on the T cells and the indirect modulation of T cell responsiveness through altered local cytokine networks [95, 97]. High pretransplant levels of HGF are also associated with a reduced risk of severe GVHD [98], and animal studies suggest that this cytokine has immunosuppressive effects in allotransplantation [99, 100].

The cytokine system constitutes an interacting functional network [58, 88], and it may therefore be more relevant to investigate cytokine profiles rather than single cytokines. In a recent study we investigated the associations between pretransplant cytokine profiles and the risk of post-transplant complications [98]. The use of hierarchical clustering allowed us to identified three distinct patient subsets based on the pretransplant serum profile of 35 cytokines (*table 3*) [98]. One of these subsets was characterized by a low rate of severe, acute GVHD and early death after transplantation, and the cytokine profile of these low-risk patients was characterized by high levels of potentially immunosuppressive HGF and G-CSF and, in addition, high fibroblast growth factor (bFGF) levels and low levels of the GVHD-associated soluble tumor necrosis factor receptor 1 (TNFR1) [98]. Thus, the pretransplant cytokine serum profile reflects the influence of pretransplant factors on the risk of early post-transplant mortality These pretransplant profiles seem to reflect the specific biological

Table 3
The serum cytokine profile in patients with acute myeloid leukemia; a summary of the results from previous studies comparing the systemic cytokine profiles in AML patients with the cytokine profiles in healthy individuals [88, 89, 93]. ↑ and ↓ indicate increased or decreased values respectively, compared to healthy controls.

Cytokine	Cytokine serum profiles before and following antileukemic treatment			
	Prechemotherapy profile [89]	**Prechemotherapy profile [88]**	**Pre-allotransplant/Complete remission profile [98]**	**Post-allotransplant profile [98]**
Patient number	82 AML	162 AML 100 MDS	27 AML 13 ALL 4 Other	27 AML 13 ALL 4 Other
Median age (range)	67 (27-90)	62 (21-86)	47 (18-61)	47 (18-61)
Chemokine				
CCL2	-	-	-	↑
CCL3	-	↓	↓	↑
CCL4	-	-	-	↑
CCL5	-	-	nt	nt
CCL11	-	-	↑	↑
CXCL5	↓	nt	↓	-
CXCL8/IL-8	↑	↑	↑	↑
CXCL10	↑	↑	-	↑
CXCL11	-	nt	↑	↑
Interleukins				
IL-1α	↓	nt	-	-
IL-1β	-	-	-	-
IL-1RA	-	↑	↑	↓
IL-2	↓	-	↓	↑
IL-4	↓	↓	↓	-
IL-5	↑	-	nt	nt
IL-6	↑	↓	↑	-
IL-7	nt	↓	nt	nt
IL-9	nt	-	nt	nt
IL-10	↑	↓	-	↑
IL-12	-	↑	nt	nt
IL-13	-	-	nt	nt
IL-15	nt	↑	nt	nt
IL-17	-	-	↑	-
Growth factors				
EGF	↑	nt	-	↑
bFGF	-	-	nt	nt
HGF	↑	nt	-	↑
G-CSF	↑	-	↑	↑
GM-CSF	↑	↑	↑	-
Leptin	-	nt	-	-
PDGF-BB	nt	↓	nt	nt
TPO	-	nt	-	↑
VEGF	-	-	-	-
Immunoregulatory cytokines				
IFNγ	↑	↓	↓	↓
TNF-α	-	-	↓	-
CD40-Ligand	-	nt	↑	↑

nt = not tested.

status of AML patients in complete hematological remission because they are different both from the profiles described in untreated AML, patients with other hematological diseases (MDS and aplastic anemia) and from the profiles detected three to six months after the allotransplantation (*table 3*) [88-90].

Other pretransplant factors are also associated with an increased risk of post-transplant complications, *e.g.* liver pathology, the use of total body irradiation (TBI) in pretransplant conditioning, and the extent of pretransplant chemotherapy. The TBI effect may at least partly be caused by a transient activation of remaining host dendritic cells and thereby presentation of alloantigens to donor-derived immunocompetent cells [95]. A predominantly immunosuppressive, pretransplant cytokine profile may then reduce the impact of these effects. Future studies should therefore try to clarify whether this altered pretransplant cytokine network is only a marker or an effector mechanism directly involved in the development of severe, post-transplant complications.

THE SYSTEMIC CYTOKINE PROFILE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION – CYTOKINE-MEDIATED EFFECTS ON ANGIOREGULATION AND IMMUNOREGULATION

Cytokines are key regulators of post-transplant, hematological and immunological reconstitution, defense against infections and acute and chronic GVHD. The development of GVHD seems to be a multistep process involving cytokine release by sequential activation of monocytes and T cells [101], and this is probably mediated and/or followed by a general cytokine storm [95]. This observation also illustrates the importance of studying broader cytokine profiles rather than single cytokines. However, some cytokines seem to be of particular importance and to have a different impact depending on the time of evaluation [93]; this is true for HGF, where high pretransplant levels seem to be associated with a decreased risk of acute GVHD and early death [98-100], whereas high, post-transplant levels are associated with increased risk of acute GVHD [102, 103]. Currently, there is no validated, diagnostic biomarker for chronic GVHD with diagnostic, prognostic or predictive value [104]. We compared the pre- and post-transplant cytokine profiles in 26 patients tested three to six months after allo-SCT. The post-transplant profiles differed from healthy controls and thus did not represent a normalization of the systemic cytokine network [98]. The most striking difference was a late, post-transplant increase in several chemokines [98]. Furthermore, Paczensky *et al.* screened a biomarker panel to distinguish acute GVHD (a distinct clinical picture developing during the first 100 post-transplant days) from non-GVHD complications after allo-SCT [102]. They detected 35 biomarkers that differed significantly between patients with and without acute GVHD. When using a validation group of 424 patients and logistic regression analysis, they detected four biomarkers that could be used to identify patients with GVHD, *i.e.* IL-8/CXCL8, TNFR1, HGF and IL-2Rα [102]. All four markers had previously been associated with the development of GVHD [103, 105-107], and the combined use of these markers increased the diagnostic potential compared with the use of single markers [101]. These observations further support the hypothesis that cytokine profiles can be used for prognostic and diagnostic evaluation of allotransplanted patients

AUTOLOGOUS STEM CELL TRANSPLANTATION – ANGIOREGULATORY EFFECTS OF STEM CELL MOBILIZATION AND HARVESTING

Autologous stem cell transplantation can be used in the post-remission consolidation treatment of AML [14]. The angioregulatory cytokine network has not been investigated in detail in AML patients receiving auto-SCT, but a recent study examined the effects of peripheral blood stem cell harvesting on serum cytokine levels in patients with multiple myeloma (MM); these observations may also be relevant in AML [108]. This study showed that the stem cell harvesting procedure altered the plasma cytokine profile with (i) a significant and relatively large increase in HGF levels immediately after stem cell apheresis, this increase persisting for more than 24 hours; (ii) the apheresis decreased Ang-1 and VEGF levels but increased the levels of endocan which is a microvascular endothelial cell marker; (iii) the procedure did not alter the levels of Ang-1, Ang-2, VEGF, angiogenin or bFGF. These observations suggest that stem cell harvesting can alter angioregulation, but it is not known whether similar alterations would have any clinical impact in AML.

THE CYTOKINE NETWORK IN HUMAN AML - ANGIOREGULATION, IMMUNOREGULATION AND FUTURE ANTILEUKEMIC THERAPY

Angioregulatory cytokines seem to have important direct and indirect effects in the regulation of AML cell proliferation, and the use of cytokine inhibitors in the treatment of AML is therefore considered. In contrast to other hematological malignancies where antiangiogenic drugs are used in their routine treatment (*e.g.* bortezomib, thalidomide and lenalidomide treatment in myeloma [109]), the use of such drugs in the treatment of AML is not established, even though several antiangiogenic strategies are now being investigated in preclinical studies and clinical trials of human AML (*figure 1*).

Direct targeting of angiogenic factors – results from clinical studies involving single agent therapy

Bevacizumab is a VEGF-A-specific, recombinant, monoclonal antibody that had a limited clinical effect in two studies including patients with refractory AML [110, 111]. Sunitinib is a VEGF RTK inhibitor that, in addition, inhibits KIT and FLT3-initiated signaling. This drug also seems to have a limited effect in AML with complete or partial remissions of short duration, but only for a minority of patients [112, 113]. HGF/MET inhibitors [72] and targeting of Ang/Tie2 [114] have not been examined in clinical AML studies. MMP inhibitors have also been considered as possible targets in antiangiogenic therapy [82], but so far they have only been tried in the treatment of solid tumors with initial results having been disappointing [82]. Thus, antiangiogenic therapy alone seems to have limited antileukemic effects and future clinical studies in human AML should rather focus on combination therapy, *e.g.* combination with conventional intensive therapy or new targeted therapy.

Therapeutic targeting of endothelial cells – vascular disrupting agents

Another possible strategy is to destabilize cancer-induced microvessel networks through selective targeting of proliferating endothelial cells [115]. Vascular disrupting agents target microvessels by direct binding to microtubules in endothelial cells; the intention is then to cause a rapid and selective vascular shutdown in the malignant microenvironment and thereby induce secondary cancer cell death due to ischemia [115]. The effects of these agents seem to be, at least, partly mediated through inhibition of the tubulin skeleton [115]. The possibility of systemic toxicity due to general endothelial cell damage or endothelial

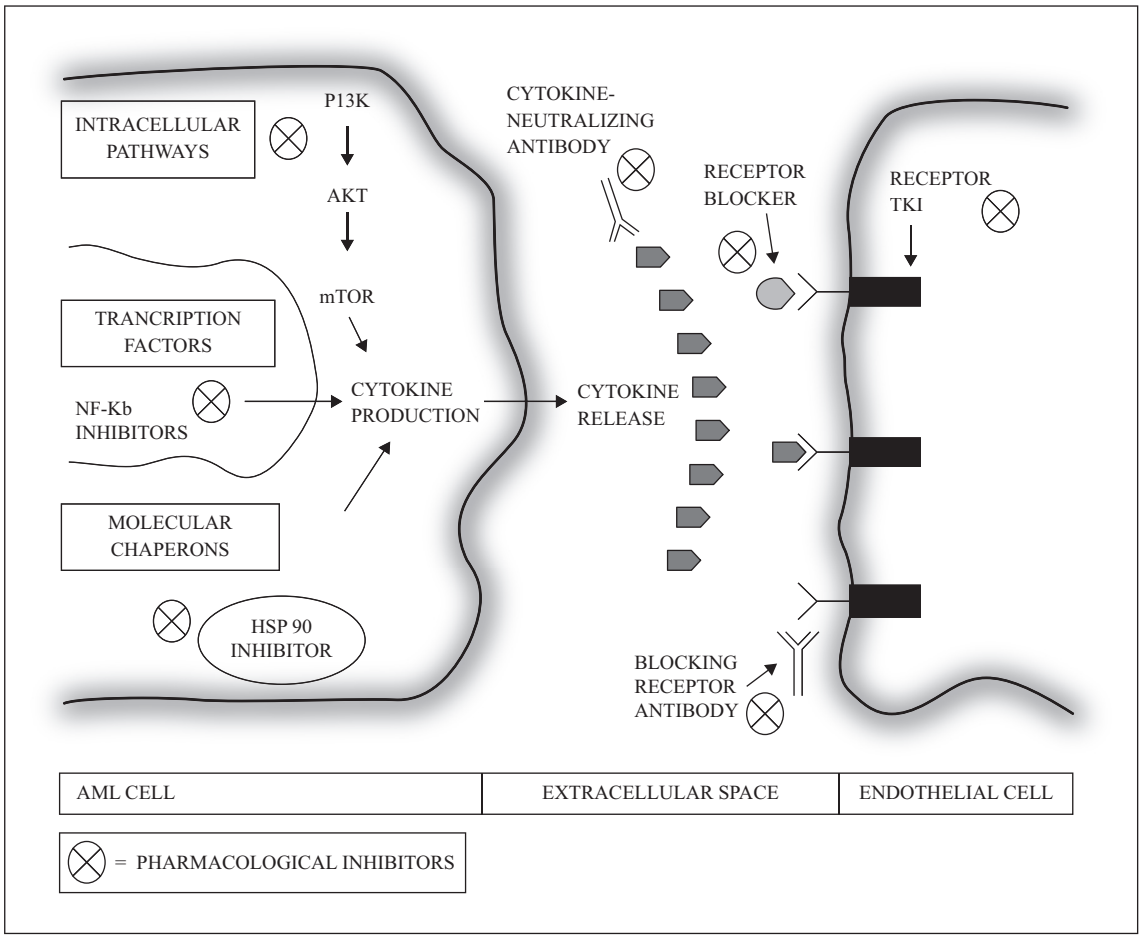

Figure 1

Cytokine-mediated crosstalk between endothelial and AML cells; possible strategies for targeted therapy. The figure illustrates the crosstalk, and the different strategies to inhibit this system. Various cytokines are released by AML cells into the extracellular space. This release is mediated through aberrant activation of intracellular signaling systems, including (i) the intracellular signaling pathway PI3K-AKT-mTOR, (ii) the transcription factor NF-κB and (iii) the molecular chaperone HSP90. All these system can be inhibited by specific inhibitors. Once released into the extracellular space, cytokines can be inhibited by several approaches, including directly binding of neutralizing antibodies, specific blocking of cytokine receptors, binding of receptor-blocking antibodies and receptor TKI inhibitors.
Abbreviations: PI3K, phosphatidylinositol 3 kinase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor κB; HSP90, heat shock protein 90, TKI, tyrosine kinase inhibitor.

dysfunction needs to be further investigated [116], but fosbretabulin/CA4P [115] and its analogue OXi4503 have shown promising preclinical results [29, 117]

Inhibition of cytokine-dependent angiogenesis through targeting of intracellular signaling

Pharmacological targeting of intracellular signaling offers the possibility to inhibit the AML cells as well as AML-supporting stromal cells, including endothelial cells [118]. Several strategies are considered and we will discuss NF-κB, heat shock protein (HSPs) and PI3K-mTOR inhibition (*figure 1*). NF-κB is a group of transcription factors involved in several processes that are important in leukemogenesis, including inflammation, angiogenesis and cytokine release [119]. The NF-κB system in leukemic cells can be modulated by extracellular factors, *e.g.* TNF-α release by neighboring immunocompetent cells [120] or by a hypoxic bone marrow microenvironment that leads to local chemokine release [119] that will affect cell trafficking in the leukemic bone marrow. Furthermore, the chemokine receptors CXCR4 and CX3CR1 become up-regulated in primary human AML cells during conventional *in vivo* chemotherapy even though IKK/NF-κB cascade genes become down-regulated [121]. In addition, systemic chemokine levels are also altered in AML patients during/following conventional chemotherapy [84]. Similar effects to conventional chemotherapy with increased chemokine release together with decreased CXCR4 expression by primary AML cells, but with increased levels of active NF-κB, have also been observed after *in vitro* treatment with the proapoptotic protein kinase C agonist Ingenol-3-angelate (PEP005) [122]. Finally, several other angiogenic factors (*e.g.* VEGF, IL-8/CXCL8 and MMP-9) are also regulated by NF-κB and their constitutive release by malignant cells is thereby suppressed by NF-κB-inhibition [82, 123-126]. Even though a detailed understanding of the molecular mechanisms of thalidomide, lenaladomide and bortezomib in AML is not available, these drugs may inhibit angiogenesis through NF-κB inhibition in myeloma treatment [109, 119], and similar effects may be operative in AML [119, 127].

Heat shock proteins (HSPs) are a group of proteins that show low expression under physiological conditions, but increased levels are triggered during environmental stress, *i.e.* malignant transformation [128]. HSPs are usually cytoplasmic proteins that can be released extracellularly as soluble, biologically-active forms [129, 130]. They

function as molecular chaperons and are important for the stabilization of several client proteins. HSP90 seems especially important in the regulation of cell proliferation, survival and adaptation to unfavorable microenvironments [128]. HSP90 inhibition probably targets multiple proangiogenic regulators and may thus have direct, inhibitory effects on leukemic cells and additional, indirect antileukemic effects through inhibition of angiogenesis by the targeting of client proteins [75, 128, 131-133]. Firstly, the HIF/VEGF axis is usually up-regulated during angiogenesis [134], and several key mediators of this pathway, including hypoxia inhibitory factor (HIF) and the VEGF receptor, are HSP90 client proteins [131]. Secondly, HSP90 is important for the stabilization of AKT; this kinase mediates phosphorylation and activation of NO synthase (NOS) that is important for the activation of endothelial cells [131]. Thirdly, HSP90 inhibition can reduce the constitutive release of several proangiogenic factors by human AML cells [132], and the HSP90 inhibitor SNX-2112 suppresses endothelial cell organization into capillary tubes through inhibition of AKT/NOS [135]. Clinical trials of HSP90 inhibitors in AML are in progress [136, 137]; it will then be important to evaluate the contribution of both the direct and indirect antileukemic effects during *in vivo* treatment [133, 138].

The PI3K-mTOR pathway seems important in angiogenesis: (i) PI3K activation and phosphorylation is probably important for VEGF receptor activation [139]; (ii) Ang1 can phosphorylate and activate Tie2 in a PI3K-dependent manner and survival, and migration of endothelial cells is thereby induced [139], and (iii) mTOR seems important for angiogenesis by acting as a switch in endothelial cell metabolism and their proliferation is thereby supported [140]. Thus, PI3K-mTOR inhibition seems to mediate antiangiogenic effect through several molecular mechanisms [141]; similar effects are seen in AML [142] and an alteration of local angioregulatory cytokine profiles seems to be important [143].

Targeted therapy after remission induction through modulation of the cytokine network

New targeting therapies with antiangiogenic effects are currently being considered in human AML. The clinical experience so far is limited, but the scientific basis for simultaneous inhibition of several intracellular signaling systems with effects on both AML and endothelial cells is emerging. Whether these strategies should be incorporated in consolidation chemotherapy or as a post-transplant maintenance treatment remains to be clarified. It may also be possible to combine such strategies with conventional chemotherapy, but future studies will be needed to clarify this question. The question of post-remission treatment is difficult especially in elderly patients where the value of intensive consolidation treatment has been questioned and two-cycle regimens have thus been developed [144]. However, even for the patients included in this last study, with a relatively low median age of 69 years, treatment-related mortality was 10%. Another study has compared an intensive strategy consisting of a single, intensive consolidation course versus a prolonged, but less intensive outpatient strategy; the decreased intensity regimen showed improved survival with decreased mortality [145]. Cytokine targeting including antiangiogenic strategies should therefore be investigated further, in addition to long-time maintenance treatment, especially for the large group of elderly patients.

CONCLUDING COMMENTS

AML is an aggressive malignancy; even younger patients, who can receive comparatively high levels of intensive chemotherapy, have a relatively low, long-term, disease-free survival. For the large group of elderly patients affected, intensive strategies are not possible because of high, treatment-related mortality. Cytokine-directed, antiangiogenic therapy may be tried in order to increase the antileukemic effects of conventional intensive chemotherapy. This would serve as a possible low-toxicity treatment in elderly, AML patients or as a post-transplant immunomodulatory/antileukemic strategy.

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