INTRODUCTION

AML is an aggressive disorder characterized by accumulation of immature malignant cells in bone marrow. Most adult patients with AML die from the disease. Even high-dose multi-agent chemotherapy and allogeneic stem cell transplantation often fail to prevent relapses. The heterogeneous phenotype of AML is based on cytogenetic mutations and molecular aberrations. Based on analysing large cohorts of patients, most have a defined prognostic significance with differences. Based on assessing cell response to changing levels of cytokine receptor, signal transduction pathways (STP) are activated. Abnormalities in signalling through STP are common in AML. In fact, autonomous cell proliferation during leukemogenesis is unlikely without altered STP activation. The frequency of STP activation in AML (ref.12) exceeds the frequency of mutations and genetic alterations in receptors or STP components suggesting that STP activation, triggered by cytokines binding to unmutated receptors, is a frequent event in leukemia development and should not be underestimated. Aberrant cytokine levels in AML and abnormal responsiveness to them is well-documented. The overexpression of cytokines in leukemia patients declines in complete remission, suggesting that these events are dependent on AML activity, possibly due to autonomous blast cytokine secretion. Several factors have been reported to contribute to the growth advantage of the malignant clone, including the autonomous proliferation and autocrine production of cytokines by these cells, such as G-CSF, GM-CSF, IL-1 and IL-6 (ref.17-19). Based on attempts to further classify AML patients according to cell response to changing levels of chemokines, new classification schemes of AML have been developed. These also reflect the ability of AML cells to stimulate angiogenesis or chemotaxis. It remains to establish whether this kind of classification for different cytokine families, especially interleukins, is of prognostic importance.
value in AML risk stratification. This would provide us with deeper insight into contributing factors triggering blast cell proliferation, migration and tissue infiltration as these are the basic questions in AML cell biology with possible treatment consequences.

### CYTOKINES IN AML PROLIFERATION AND PROGNOSIS

Based on their biological effects, cytokines may be divided into six major families\textsuperscript{21} - interleukins (IL), chemokines, interferons (INF), tumor necrosis factors (TNF), growth factors of hematopoiesis and transforming growth factor-β (TGF-β) family members. For further details see Table 1.

Cytokine levels at AML diagnosis are aberrant and normalize in remission\textsuperscript{15,16}. Levels of circulating cytokines and changes in patient outcome have been the subject of numerous studies. It has been reported that cytokine stimulation causes abnormal responsiveness in leukemic blasts and that leukemic blasts are often a source of cytokine or chemokine production possibly triggering autocrine and paracrine loop activation.

There are a large number of cytokines circulating in the plasma with various possible effects on AML blast cell proliferation. The most troublesome for assessing their effects is that cytokines are produced and act simultaneously, with a partially overlapping spectrum of biological effects due to frequent receptor sharing. This makes it difficult to predict the relationship between leukemogenesis contribution and individual cytokine levels.

The IL-1, IL-2 and β-chemokine of CC subgroup CCL-3 (CCL-ligand-3) have been shown to stimulate leukemic cell proliferation\textsuperscript{22,23}. To further assess the role of several single cytokines on the proliferation of patient-derived AML cells, a cytokine-induced proliferation study was performed\textsuperscript{24}. In the total study population, only autonomous blast cell proliferation was a significant predictor for prognosis from the multivariate analysis. Assuming that especially for the intermediate-risk AML patients, a further prognostic classification would have significant benefit, the AML blasts of FLT3-ITD (FMS-like tyrosine kinase 3 internal tandem duplications) negative patients with intermediate risk cytogenetics were further studied. The strongest responses to cytokine stimulation were observed for IL-3, GM-CSF and G-CSF, but only the responses to IL-1α and M-CSF were found to be predictive.

### Table 1. Basic classification of cytokines.

<table>
<thead>
<tr>
<th>Cytokine family</th>
<th>Functional subgroups</th>
<th>Physiologic effect</th>
</tr>
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<tbody>
<tr>
<td>Interleukins</td>
<td>IL-3, IL-7, Flt3-ligand</td>
<td>- stimulate hematopoiesis</td>
</tr>
<tr>
<td></td>
<td>IL-1, IL-6</td>
<td>- pluripotent, inflammatory</td>
</tr>
<tr>
<td></td>
<td>IL-2, IL-4, IL-5, IL-12, IL-13</td>
<td>- regulate T and B cell cooperation</td>
</tr>
<tr>
<td>Chemokines ( \alpha ): CXC</td>
<td></td>
<td>- regulate migration of granulocytes and lymphocytes</td>
</tr>
<tr>
<td></td>
<td>( \beta ): CC</td>
<td>- promote angiogenesis and inflammation</td>
</tr>
<tr>
<td></td>
<td>( \gamma ): C, ( \delta ): CX3C</td>
<td>- regulate migration of monocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- enable medullar homing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- regulate migration of lymphocytes</td>
</tr>
<tr>
<td>Interferons Type I: INF ( \alpha ), ( \beta ), ( \omega )</td>
<td></td>
<td>- antiviral immunity</td>
</tr>
<tr>
<td></td>
<td>Type II: INF ( \gamma )</td>
<td>- anti-proliferative effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- antitumorous activity</td>
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<tr>
<td></td>
<td></td>
<td>- response to intracellular pathogens</td>
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<tr>
<td>Tumor necrosis factors</td>
<td>TNF( \alpha ):</td>
<td>- pro-inflammatory pyrogenic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- activates non-specific immunity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- adhesive molecule expression on endothelial surface</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- may cause apoptosis -</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- overproduction causes SIRS</td>
</tr>
<tr>
<td></td>
<td>TNF( \beta ):</td>
<td>- similar in effect, but produced by T- and B lymphocytes</td>
</tr>
<tr>
<td>Colony Stimulating Factors</td>
<td>G-CSF, GM-CSF, M-CSF</td>
<td>- stimulate proliferation and maturation of myeloid precursors</td>
</tr>
<tr>
<td></td>
<td>Erythropoietin</td>
<td></td>
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<tr>
<td></td>
<td>Thrombopoietin</td>
<td></td>
</tr>
<tr>
<td>TGF-( \beta )</td>
<td></td>
<td>- stimulate growth of fibroblasts and extracellular matrix production</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- lead to MMPs inhibition</td>
</tr>
</tbody>
</table>

Modified from: (ref. \textsuperscript{21})
for therapeutic outcome. In this study, the response to IL-7, IL-11 and TNF-α could be interpreted as inhibition of AML blast cell proliferation. IL-7 is a major cytokine in lymphopoiesis and IL-11 plays a role particularly in thrombopoiesis, but the response to TNF-α stimulation in this study remains confusing. In a different study, within AML and high risk myelodysplastic syndrome (MDS) patients, a close correlation between TNF-α level and leukocyte count was found. Lower TNF-α levels were associated with higher CR rates, better overall and event-free survival. Higher TNF-α levels were statistically significant when leukocyte count was excluded from the models, confirming their predictive value for therapeutic outcomes. Higher serum TNF-α levels further correlated with higher levels of β₂-microglobulin, creatinine and alkaline phosphatase, and inversely with creatinine clearance and albumin levels. Higher TNF-α levels were also correlated with poorer performance, M4-M5 AML subtypes according to French-American-British (FAB) classification and the presence of infection.

Further, although the IL-3-receptor expression within the cytokine-induced proliferation study was not measured and thus the data on IL-3R density are not available, the non-significance of IL-3 stimulation response is an issue. As cytokine receptor expression is inducible, we can expect sufficient expression of this receptor during the cytokine induced 7 day proliferation assay. The density of IL-3Rα subunits (CD 123) on AML blasts was found to be an independent risk factor for AML (ref.32). At the clinical level, a significant correlation between the level of IL-3R expression and the number of leukemic blasts at diagnosis was observed. Patients exhibiting elevated IL-3R levels had lower complete remission rates and survival duration than those with normal IL-3R levels. Following IL-3R stimulation, various STPs, including MAPK (mitogen activated protein kinase) MEK/ERK (ref.33), phosphatidylinositol 3-kinase (PI3K) (ref.34), protein kinase A (PAK) (ref.35) and signal transducer and activator of transcription 5 (STAT-5) are activated. IL-3 mediated activation of STAT-5 up-regulates the antiapoptotic protein Bcl-xL (ref.36). The importance of STAT-5 activation is not restricted to IL-3R downstream processes. STAT-5 and causes IL-3 independent expression of antiapoptotic protein Bcl-xL.signaling was associated with coexpression of oncogenes of different molecular subgroups in LSCs and in the sample of 201 patients, it was restricted to those with the poorest prognosis.

Given its influence on cell proliferation (and leukemogenesis), it follows that STAT-5 activation needs to be regulated properly. To provide greater insight into complex processes affecting the degree of STAT-5 activation, the murine IL-3 dependent BaF3 cell line (BaF3 cells lack expression of gp 130, a common receptor subunit of the class I - IL-6 subfamily cytokine receptor, for further details see below) was investigated. RhoH is a constitutively active member of the family of Rho GTPases. Its expression is restricted to the haematopoietic lineage where it serves as a positive regulator for T-cell selection and mast cell function and, as a negative regulator for growth-related functions in other lineages. RhoH regulates IL-3-induced signalling through modulation of the activity of STAT proteins. The overexpression of RhoH decreases IL-3-induced proliferation and the activity of STAT-5. The surface expression level of the IL-3 receptor α-chain (CD123) is inversely correlated with the expression levels of RhoH. In RhoH-deficient cells, the STAT-5-dependent gene for interferon regulatory factor-1 (IRF-1) was up-regulated, leading to an up-regulation of CD123 expression. Interestingly, only BaF3 cells that overexpressed RhoH were able to activate STAT1 after stimulation with IL-3. Activation of STAT-1 is known to coincide with cell cycle arrest or apoptosis and the STAT-1 dependent cell cycle inhibitors p21Cip1 and p27Kip1 were shown to be up-regulated due to RhoH overexpression. As mentioned, elevated CD123 expression in AML patients contributes to increased proliferation of leukemic blasts, hyper-activation of STAT-5 and poor prognosis. Low expression levels of RhoH were also described as another factor in poor patient prognosis. These data demonstrate that these two findings might be connected.

Interleukin 6

IL-6 has diverse effects in malignant cell biology, with proven prognostic impact in diffuse large-cell lymphoma and chronic lymphocytic leukemia. IL-6 is a pleiotropic cytokine that can be constitutively expressed in AML cells. IL-6 levels are markedly increased in AML/MDS.
Activation of IL-6 signal transduction involves dimerization of IL-6 receptor gp130 subunit, consequently recruitment of gp130-associated protein–tyrosine kinases Jak1, Jak2, and Tyk2 and tyrosine phosphorylation of STAT-3, to a lesser extent STAT-1 (ref.49). At least two other STPs (ERK and PI3K) are also activated by IL-6 (ref.50-52). STAT-3 plays a key role in G1- to S-phase cell-cycle transition through the up-regulation of cyclins D2, D3, A, and cdc25A and concomitant down-regulation of p21 and p27 (ref.53). Constitutive STAT-3 activation has been demonstrated in AML and is described in about 20% of patients54. It was later shown that STAT-3 is constitutively phosphorylated on Tyr705 and Ser727, which could be not further up-regulated by treatment with IL-6. AML cells with constitutive STAT-3 activation also secreted high levels of IL-6 protein, suggesting probable Jak/STAT pathway stimulation in an autocrine or paracrine manner, or both55. This might lead to a growth advantage of the malignant clone, although this has not been proved. In a study on 75 patients IL-6 serum levels were not found to be predictive for CR rates, survival or event free survival27. Whether this was caused by STAT-1 activation or higher TGF-β1 serum levels remains unclear. The main function of TGF-β1 in hematopoietic cells is to regulate cell proliferation by inducing growth arrest in the G0/G1 phase of the cell cycle56,57. TGF-β1 is a member of the TGF superfamily of proteins. TGF-1 exerts its effects by binding to its receptor (TGF-receptor type II, TGF-RII), which results in recruitment of a second receptor chain (TGF-receptor type I, TGF-RI) (ref.58). Hetero-dimerization of the receptor chains leads to phosphorylation of TGF-RI, resulting in activation of its intrinsic kinase activity. SMAD (Sma- and Mad related) proteins 2 and 3 are subsequently phosphorylated by the activated TGF-RI, leading to their association with SMAD4 and translocation to the nucleus, where the SMAD2,-3,-4 complex initiates gene transcription of TGF-1-responsive genes59. To explore cross-talk between the IL-6 and TGF-1 pathways in AML blast cells, the effect of TGF-1 pre-treatment on IL-6-induced STAT-3 tyrosine phosphorylation was studied. In 10% (4 out of 40) of AML patients, a significant reduction in IL-6 mediated STAT-3 tyrosine phosphorylation after TGF-β1 pre-treatment was observed. Measured by means of SMAD3 translocation, TGF-β1 affected all of the AML cases studied, but only reduced IL-6-mediated STAT-3 tyrosine phosphorylation after pre-treatment with TGF-β1 was associated with apoptosis60. In conclusion, although 10% (4/40) is a small percentage, at least these patients could benefit from TGF-β1 treatment. Unfortunately, further specification of this group by cytogenetics or molecular genetics is not available.

CHEMOKINES

Chemokines form a cytokine family of soluble mediators with a molecular weight up to 10 kDa. Chemokines (the name of this group comes from origin chemotactic cytokines) help to regulate cell migration, are involved in angiogenesis, cellular growth control, inflammation response development and immunomodulation. All nuclear cells can produce chemokines, which is of major importance as all nuclear cells can take part in immune system activation. Chemokine secretion appears after pre-stimulation. There are four subgroups of chemokines distinguished according to the spacing of highly conserved cystein residues: C, CC, CXC, CX3C. With an overlapping spectrum of action and some kind of receptor nonspecificity, chemokines form a network redundant in count but this redundancy provides robust outputs of network activation61,64-65. In a complex study by Bruserud, the effects of various chemokines on proliferation of patient-derived AML cells and constitutive chemokine release by primary AML cells have been investigated in vitro in AML cells derived from 68 consecutive patients29. Exogenous chemokines usually had no effect on AML blast cells proliferation, but having the hematopoietic growth factors (IL-3 + GM-CSF + stem cell factor - SCF) added to the cultures, proliferation in suspension cultures occurred and specific patient subsets were identified. In nearly one third of patients’ AML cell samples, chemokine stimulation induced proliferation or led to divergent effects on proliferation. Further, the AML cells were found to release chemokines of homeostatic and inflammatory groups, and of both pro-angiogenic, with the highest levels detected for CXCL8, and the anti-angiogenic group. In contrast, some chemokines, including CXCL12 (acting on CXCR4, for more information see below) were not detected in any, or only in a few patients. The influence of chemokine release on chemotaxis was also studied. Although the patterns of chemokine release in AML cell samples differed, in the presence of chemokine release, chemotaxis was stimulated as a whole, with no detectable specific effect on various T-cell subsets. Based on experimental data, the patients were divided according to hierarchical clustering into subgroups for chemokine induced proliferation, autonomous chemokine secretion and chemotaxis activation. There was a significant correlation between no detectable in vitro proliferation and low chemokine release, showing that chemokines are excreted at higher rates by proliferating cells. None of these subgroups correlated with known prognostic factors, e.g. cytogenetics of FLT3 mutation status, demonstrating the heterogeneity of growth factors-dependent AML cells.

Besides the descriptive outcomes of the study mentioned above, several chemokine characteristics are noteworthy. The chemokine receptor/ligand interactions orchestrate the migration of cells to peripheral tissues66. As CXCL8 is usually secreted in highest levels, serum CXCL8 seems to partly reflect the AML cell burden67. The release of angiogenic stimuli from the AML cells is accelerated in hypoxia. In a hypoxic environment, the hypoxia inducible factor 1α (HIF-1α) expression by AML cells is increased, leading to up-regulation of angiogenesis-related genes and angioregulatory cytokine expression68. AML cells cultured in vitro with 1% O2 showed increased release of several CCL (CCL 3,4,5,7,8) and CXCL (CXCL1 and proangiogenic CXCL8) chemokines, vascular endothelial growth factor (VEGF) was also secreted at higher rates, compared to the same samples cultured at 21% O2. The
wide variation in cytokine expression between patients, observed in previous studies, persisted. The angiogenic effect of AML cells is, except for cytokines, also mediated by angiopoietin-1 (Ang-1) and by the matrix metalloproteinases (MMPs). Ang-1 is an AML cell constitutively released agonist on Tie-2 receptor with various interactions and possible therapeutic influence of this system. MMPs are zinc-dependent endopeptidases able to degrade all components of the extracellular matrix (ECM). The ECM cleavage leads to removing physical barriers and prepares a new space for angiogenesis. The AML cells often show constitutive release of several MMPs together with proangiogenic cytokines, which rapidly leads to angiogenesis and possibly facilitates extramedullar spread of the AML (ref.79).

**CXCL12/CXCR4 interaction in AML**

The best investigated single chemokine (not only in AML) is CXCL12. CXCL12 (often called stromal-derived factor 1α, SDF-1α) is a homeostatic chemokine constitutively secreted by marrow stromal cells. SDF-1α binds to CXCR4. This interaction allows it to retain hematopoietic progenitors and leukemia cells inside the bone marrow and allows a high persistence of leukemia cells in the bone marrow. Binding of SDF-1α to CXCR4 leads to receptor phosphorylation, triggering prolonged activation of ERK and PI3K pathways, which promotes (leukemia) cell survival. The increased CXCR4 expression on the AML cells is an independent prognostic factor and a predictor of poor outcome in AML regardless of FLT3 mutation status, but if the FLT3 is mutated, the CXCR4 expression is further enhanced. Further studies revealed that SDF-1α increases human ether-à-go-go related gene 1 (hERG1) K(+) channel expression in a dose dependent manner. SDF-1α further increases expression of several genes including beta-catenin, cyclin D1 and c-myc, which is abolished when the hERG1 K(+) channels are blocked. Here the possibilities of pharmacologic interaction become obvious. The SDF-1α analogue AMD 3100, known as plerixafor, is used in mobilizing normal progenitor cells. Various SDF-1α antagonists have been investigated. The polypeptide RCP168 seems to have strong antagonistic effect on the stromal cell-induced chemotaxis of leukemic cells. Furthermore, RCP168 blocked the binding of anti-CXCR4 monoclonal antibody 12G5 to surface CXCR4 in a concentration-dependent manner and inhibited SDF-1alpha-induced AKT and extracellular signal-regulated kinase phosphorylation. Equivalent results were obtained with the small-molecule CXCR4 inhibitor AMD3465, a second generation CXCR4 inhibitor. AMD 3465 antagonized SDF-1α and stroma-induced chemotaxis and suppressed stroma activated PI3K/AKT and MEK/ERK pathways, which effectively mobilized leukemia cells and stem cells into circulation and enhanced the sensitivity to chemotherapy or FLT3-inhibitor-induced cell death. One more candidate with possible treatment consequences is E-4031, which can induce G0/G1 arrest, impair SDF-1α induced proliferation or even induce apoptosis of AML cells. From the above data, it is obvious that SDF-1α/CXCR4 interaction plays a key role in AML development and therapeutic outcome. Modulating this interaction is a possible therapeutic approach. As the SDF-1α receptor inhibition sensitizes the AML cells to chemotherapy, it is presumed to reach either standard outcomes with less intense chemotherapy and decreased toxicity, or even better outcomes without further damaging the patients by more aggressive treatment regimens. By far the most important advantage of this possible approach is the ubiquitous expression of CXCR4 in cells populating bone marrow, which would allow us to use this strategy in various haematological malignancies.

**CYTOKINES IN ALLOGENEIC BONE MARROW TRANSPLANTATION**

Bone marrow transplantation (BMT) is a sophisticated procedure of replacing the patient’s hematopoiesis by donor graft. The process of engraftment is substantially dependent on graft pluripotent cell stimulation by various cytokines and the repopulation of bone marrow. It also assumes sufficiently mature cell production, as well as normal hematopoiesis, is possible due to proper cytokine interplay. The aim of further cytokine level study in pre- and post-transplant period is to recognize groups of patients with either better outcome and lower risk of complications, or higher risk groups in terms of graft rejection or GvHD development. The cytokines are abundant and functionally diverse. For this reason, it is not easy to describe the integrative effect of cytokines on BMT outcome. According to pre-transplant cytokine profile investigations, the cytokine profiles of patients undergoing BMT differ from each other, and all differ from healthy controls, although all patients achieved complete remission. Briefly, three subgroups of patients according to hierarchical clustering could be identified. These groups differed especially in the hepatocyte growth factor (HGF) and G-CSF levels. One of these groups, characterized by high levels of HGF and G-CSF, showed low early treatment-related mortality.

Apropos allogeneic stem cell transplantation, a novel T-helper lymphocyte subset called Th17, with distinct effects in allotransplanted patients, has been described. This lymphocyte subset was shown to have an important role in GvHD development and AML relapse control. The Th17 cells preferably differentiate from CD4+CD161+ T-cell subset, but there are probably more pathways possible. Th17 development seems to be dependent on IL-1β signalling, supported by IL-6 and IL-23 (ref.89), with a distinct role for TGF-β (ref.89). AML cells can affect T-cell differentiation and Th17 development within the bone marrow through their release of IL-1β, IL-6 (ref.91) and T-cell chemotactic cytokines. Surprisingly, the levels of circulating Th17 cells were not confirmed to be increased in untreated AML patients, which is in contrast to a previous study, possibly due to different mean age of patients enrolled. On the other hand, Th17 cells are not depleted during conventional chemotherapy and circulating Th17 cells can be detected even in periods of severe chemotherapy-induced lymphopenia, so that suf-
ficient pre-transplant levels of Th17 cells may be expected. Th17 cells were not shown to have direct antileukemic effect in AML, but the Th17/Treg (T-regulatory cells) ratio seems to be important. Treg are immunosuppressive CD4+CD25high regulatory T-cells, high pre-treatment Treg levels or lower Th17/Treg ratio seem to be associated with adverse prognosis. Pre-transplant targeting of Treg cells or affecting Th17/Treg ratio is then a possible strategy for reducing the overall risk of AML relapse.

Th17-lymphocyte subset coordinates and regulates local inflammation through IL-17 release. The biological function of Th17 in allotransplanted patients is to facilitate GvHD development. Genetic variants in the IL-23/Th17 pathway, based on gene polymorphism, have influence on both infectious and immunological posttransplant complications. Secondly, high levels of IL-17 during early posttransplant cytopenia were observed in patients who later developed acute GvHD (ref.99). This was quite a small study and hence the results should be interpreted with caution, but this observation supports the results of the previous study highlights the role of IL-17 in GvHD development. Further, patients receiving higher dose Th17 cells in the bone marrow graft or a higher dose Tc17 cells in the PBSC graft exhibited increased incidence of acute GvHD. Increased levels of Th17 cells were also observed at the onset of acute GvHD and these levels normalized when patients responded to treatment. However, the necessity of Th17 cells for GvHD development is not absolute in GvHD pathogenesis. Th17 cells interact with other T-cell subsets and the relative importance of distinct Th subsets seems to differ between various organs, possibly due to organ-specific variation in the chemokine network and Th subsets-specific chemotactic receptor expression. These observations suggest that Th17 cells are important in GvHD development. As the IL-17 itself has only minor anti-proliferative effect on AML blasts, which was observed only in a minority of patients, its role seems to be initially in immunomodulation. The results mentioned above indicate, that Th17 cells enhancement should be considered at high risk of AML relapse with no clinical GvHD, whereas Th17 cells inhibition or depletion may be useful in treatment of excessive GvHD.

CYTOKINES IN AML THERAPY

The effects of various cytokines on AML cell proliferation and survival have been tested. Logically, cytokines with major impact on AML cell proliferation or apoptosis are candidates for therapeutic administration. IL-2 and the IL-2 in combination with histamine dihydrochloride were tested in randomized maintenance therapy trials. IL-2 alone was not found to be an effective remission maintenance therapy for AML patients in first CR. The combined immunotherapy of IL-2 with histamine dihydrochloride significantly improved leukemia free survival, but the overall survival was not improved. The data of this study were later reassessed and the consistency and robustness of the study were confirmed. Leukemia free survival was offered as an acceptable surrogate for overall survival. The patients in these trials were randomized according to complete remission attainment or demographic parameters, not according to STP activation, cytokine receptor expression etc., which does not fully reflect the AML heterogeneity. The disclosure of exact STP activation or further molecular mechanisms at the time of therapy initiation is not routinely available. This seems to be the main disadvantage of cytokines usage in AML treatment. The high activity of the disease often calls for urgent treatment initiation, so that the usage of suitable cytokines in AML treatment is not possible earlier than during the consolidation phase, when each cytokine, even if leading to massive AML burden reduction, would have only narrow spectrum of use. In particular cases, the AML cell growth is independent upon cytokine stimulation. On the other hand, there are chemokine-mediated processes affecting all cells participating in hematopoiesis or immune response control, namely SDF-1α/CXCR4 interaction in bone marrow populating cells or Th17 cell activity in immune response, GvHD development and relapse control. Inhibition of leukemia mediated angiogenesis or MMPs inhibition is a possible addition to treatment of extra-medullary spreading leukemias, which are difficult to treat, show inferior outcomes and occur more commonly after allogeneic stem cell transplantation, as the graft versus leukemia effect seems to be stronger in bone marrow than in the peripheral tissues. Also some signalling pathways, for example STAT-5, have major impact on leukemia cell proliferation and are commonly activated in AML with various genetic alterations.

We do believe that various modulations of these interactions will find therapeutic application. There is still much to investigate and describe, until the cytokine network modulation or specific STPs inhibition become an integrated part of post-remission therapy but if successful, it would greatly improve AML therapy outcomes. While the standard chemotherapy regimens have reliable outcomes in the majority of cases, cytokine network modulation would provide increased selectivity of treatment with the potential of further improvement in disease control and treatment-related toxicity reduction.

CYTOKINE RECEPTORS AND THEIR PROGNOSTIC SIGNIFICANCE

The prognosis of AML patient is based on the interaction of treatment, the ability of AML to survive, and factors like increased risk of infections and life threatening bleeding. The survivability depends on conditions triggering cell division or providing the cell with an antiapoptotic phenotype and hence drug resistance. Cytokines are involved in intercellular communication, capable of providing target cells with proliferation inducing signals and protect it against apoptosis. They act through binding to surface receptors, that, based on the structure and STP activation mechanisms, may be divided into different families. Class I hematopoietic cytokine receptors are multimolecular complexes of different receptor subunits...
α, β and χ. According to transmembrane subunit, IL-2, erythropoietin receptor (EPOR), also called IL-3 and IL-6 subfamilies are distinguished. The IL-2 receptor subfamily is sharing χ (CD 132) subunit and binds IL-2, IL-4, IL-7, IL-9, IL-13, IL-15 and IL-21. The IL-3 receptor subfamily carries βγ (CD131) subunit and binds IL-3, IL-5, GM-CSF and EPO. The IL-6 receptor subfamily is sharing β chain gp130 (CD 130) and binds IL-6, IL-11, IL-27, leukemia inhibiting factor (LIF) and oncostatin M. Stimulated class I hematopoietic receptors cause JAK/STAT signalling pathways activation. Note that IL-3, affecting mostly myeloid progenitors, and IL-7, affecting mostly lymphoid progenitors, have receptors of different subfamilies. Class II hematopoietin receptors bind interferons α, β, χ and IL-10. The class II hematopoietin receptors show structural similarity to class I hematopoietin receptors and are also coupled with JAK/STAT signalling transduction. The TNF superfamily of receptors and ligands comprises at least 30 receptors and 20 ligands. Signalling through TNF receptors may lead either to cell proliferation and inflammatory response, or may trigger apoptosis by receptor death domain activation. The chemokine receptors are G-protein coupled, bind chemokines with quite a high affinity and typical overlapping receptor specificity. The TGF-β receptor family consists of seven type I and five type II receptors, that heterodimerize to form receptors for multiple TGF-β family members. Further signalization is either SMAD dependent or independent.

In AML cell biology but not only in this disease, the receptor tyrosine kinase (RTKs) family is clinically most relevant at the moment. It comprises receptors vital for hematopoiesis and mature blood cell function. The two most important RTKs members in AML are c-Kit and FLT-3 receptor. Platelet derived growth factor receptor (PDGFR), anaplastic large cell lymphoma kinase (ALK), fibroblast growth factor (FGF) receptor, IL-1 receptor, and M-CSF receptor also belong to the RTKs family. RTKs are activated by ligand-induced receptor dimerization. The intracellular kinase domains then become activated and the receptor cytoplasmic tyrosin residues and other tethered substrates are phosphorylated. Mutations of c-Kit and FLT-3 are often found in AML with normal cytogenetics. Mutations in the c-Kit and FLT-3 receptor provide AML cell with permanent proliferative signal, protection from apoptosis, and negatively influence patient prognosis. Specific inhibition of mutated tyrosininkinases is a possible therapeutic approach. Unfortunately the results are not convincing, probably due to a high degree of internal heterogeneity.

QUALITY OF LIFE ASPECT

AML is a serious, mostly lethal disease. It often appears unexpectedly and with serious symptoms and poor prognosis. Treatment consists of aggressive, often multiagent chemotherapy. Not unexpectedly, as the AML treatment is generally disease based, the quality of life aspect is often overlooked. From the onco-hematologic point of view, the ability to promote or arrest malignant cell proliferation is of paramount importance. However, from the patient point of view, quality of life is an important aspect, and crucial for compliance. Given the long duration of treatment with a high risk of fatal complications and uncertain outcome, this aspect should not be underestimated.

Cytokine levels are increased in AML/MDS patients, initiating a pro-inflammatory and pro-proliferative environment. Inflammation-associated symptoms such as fatigue and increased body temperature bear this out. Aberrant cytokine levels are responsible for these symptoms. The TNF-α, IL-1 receptor antagonist and IL-6 levels were found to be related to ratings of fatigue while higher IL-6 levels were even associated with poorer executive functions before treatment. In both cases, these findings were not associated significantly with haemoglobin levels, which should be interpreted as evidence of the influence of a pro-inflammatory microenvironment. On the other hand, higher IL-8 was associated with better memory performance. There were 54 patients in this study, comprising both AML and MDS patients, but only 26 patients were reassessed after one month period, so that the follow-up is incomplete. When evaluated, the data available show that although the fatigue did worsen after therapy initiation, in general the treatment did not seem to have adverse effects on cognitive function. The overall quality of life was found to be acceptable but could be improved. This could also be an aspect in novel therapeutic approach evaluation. We believe that cytokine investigation will help us to define novel therapeutic approaches, allowing the treatment to be more accurate in targeting the origin of leukemogenesis, and will provide better outcomes and higher quality of life for AML patients.

ABBREVIATIONS

AML, Acute myeloid leukemia; CR, Complete remission; ERK, Extracellular signal regulated kinase; FLT3-ITD, FMS-like tyrosine kinase 3 internal tandem duplications; G-CSF, Granulocyte colony stimulating factor; GM-CSF, Granulocyte/macrophage colony stimulating factor; GvHD, Graft versus host disease; IL, Interleukin; LIC, Leukemia initiating cell; LSC, Leukemia stem cell; MAPK, Mitogen activated protein kinase; MMPs, Matrix metalloproteinases; MRD, Minimal residual disease; PAK, Protein kinase A; PI3K, Phosphatidylinositol 3-kinase; Raf; RAS activated factor; STP, Signal transduction pathway; STAT, Signal transducer and activator of transcription

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CONFLICT OF INTEREST STATEMENT

Author’s conflict of interest disclosure: None declared

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