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Interleukin-2 receptor alpha-chain (CD25) expression on leukaemic blasts is predictive for outcome and level of residual disease in AML

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ABSTRACT

We investigated the role of CD25 as a prognostic marker in acute myeloid leukaemia (AML).

Seventy-two newly diagnosed patients ⩽60 years were retrospectively analysed by flow cytometry for CD25 positivity of AML blasts.

Patients with CD25 expression of >10%, when compared to ⩽10%, had a significantly shorter overall survival (OS, $p = 0.0005$) and relapse-free survival (RFS, $p = 0.005$). In multivariate analysis CD25 expression is an independent adverse factor for OS and RFS. High CD25 combined with FLT3-ITD positivity resulted in the poorest OS and RFS ($p = 0.001$ and $p = 0.003$, respectively). CD25 expression remained prognostic within the intermediate cytogenetic risk group. In addition, after the first cycle of chemotherapy, a significantly higher MRD frequency was found in patients expressing CD25 above cut-off ($p = 0.003$).

Our results show that CD25 expression is an independent adverse prognostic marker in AML patients ⩽60 and correlates with MRD.

1. Introduction

Acute myeloid leukaemia (AML) is characterised by a block in differentiation resulting in an accumulation of immature cells in the bone marrow (BM) and is generally treated with high-dose chemotherapy. Although 70–80% of patients achieve complete remission (CR), relapses occur frequently which lead to four years overall survival of only 30–40%.[1] Relapse is thought to emerge from the outgrowth of remaining malignant cells after chemotherapy, further referred to as minimal residual disease (MRD). Various parameters present at diagnosis were found to correlate with MRD cell frequency and survival, including apoptosis-related parameters,[2] multidrug resistance-related parameters[3] and size of the stem cell compartment.[4] Previously, we and others showed that in turn the frequency of MRD cells after chemotherapy is of prognostic value for survival.[5–8] Apart from these, genetic markers associated with increased proliferation such as FLT3-Internal tandem duplication (ITD), pERK and pAKT have shown to be associated with poor prognosis in AML,[9,10] reflected by high MRD cell frequency.[11] CD25, also associated with proliferation, represents the α-chain of the interleukin-2 receptor (IL-2Rα), a low affinity binding receptor.[12–14] The IL-2 receptor is composed of different combinations of three subunits (α, β and γ chains) and is normally expressed on activated T-cells. Upon binding its ligand IL-2, the IL-2 receptor induces T-cell proliferation and differentiation.[15,16] Recently, Nakase and colleagues described CD25 as a poor prognostic factor in acute

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lymphoblastic leukaemia.[17] In AML, cytogenetics is used to stratify cases for appropriate risk-adapted therapy. However, 40–49% of the adult AML patients have a cytogenetically normal phenotype[18] and these patients are assigned to the intermediate risk group. In fact, the majority of AML patients (67%) are classified within the group with intermediate cytogenetic risk,[19] which would make additional prognostic factors particularly useful in this group. In search for such factors, we investigated the impact of CD25 positivity at diagnosis. For that purpose, we retrospectively investigated CD25 expression in 116 AML patients by immunophenotyping.

2. Material and methods

2.1. Patient samples at diagnosis

In the period July 2003 to February 2006, BM and peripheral blood samples were obtained from patients with AML and high risk MDS at the VU University Medical Center after informed consent. For this study, FAB M3 patients were excluded from this cohort, as well as 11 patients where the amount of material was too limited. Finally, 99 BM and 17 peripheral blood samples could be analysed for CD25 expression. 3/72 patients ⩽60 and 17/44 patients >60 were not treated with high-dose chemotherapy but were offered supportive care instead. 4/72 patients ⩽60 were treated elsewhere. Therefore, these cases were used only for correlations with diagnosis CD25 expression and other prognostic factors for AML but excluded from survival analysis. Immunophenotyping was performed on fresh diagnosis samples and clinical data were reviewed retrospectively. Normal BM was obtained after informed consent from healthy volunteers ($n = 12$). The diagnosis AML was based on morphology, cytogenetics and immunophenotyping. Patient characteristics are shown in Table 1. Additional to the freshly obtained diagnosis samples, cryopreserved diagnosis samples from 18 FLT3-ITD positive patients were thawed and immunophenotypically analysed to obtain additional data for that patient group. The procedure for sample freezing and thawing was as described before.[4] To analyse whether CD25 expression on fresh and frozen samples is comparable, six samples with low and intermediate CD25 expression were thawed and CD25 positivity on leukaemic blasts and lymphocytes was compared to the expression of CD25 in fresh material. No significant difference was found for blast and/or lymphocyte CD25 positivity in these samples ($p = 0.21$ and $p = 0.17$, respectively).

2.2. Treatment characteristics

Patients were treated according to treatment protocols defined by the Dutch-Belgian-Swiss Haemato-Oncology Cooperative Group (HOVON). AML patients ⩽60 years of age ($n = 60$) were treated according to the HOVON 42 and HOVON 42a protocols, or according to modified HOVON protocols ($n = 5$), patients >60 ($n = 27$) were treated according to the HOVON 43 protocol (see http://www.hovon.nl for treatment details). All protocols show a basically identical design, consisting of at least two cycles of remission induction therapy. Patients ⩽60 in complete remission (CR) after two cycles of chemotherapy received either allogeneic ($n = 26$) or autologous stem cell transplantation ($n = 8$) or received a third course of intensive chemotherapy ($n = 8$). Other patients did not achieve CR after two cycles ($n = 5$), died during treatment ($n = 11$) or went off protocol for other reasons ($n = 7$). CR was defined as less than 5% blasts present in BM in a state of haematological recovery (absolute neutrophil count $\geqslant 1.5 \times 10^9$/l and transfusion-independent platelet count $\geqslant 100 \times 10^9$/l).

2.3. Cytogenetics

Patients were classified in three risk groups for cytogenetic abnormalities according to Grimwade and colleagues,[19] (Table 1). Favourable risk was defined by the presence of t(8;21), t(15;17) or inv(16), while patients with either five unrelated abnormalities, monosomy 5 or 7, deletions in the long arm of chromosome 5, or 3q abnormalities were defined as poor prognosis. Patients with normal karyotype, or chromosomal abnormalities not enclosed in the favourable or poor risk groups, were defined as intermediate risk. FLT3 internal tandem duplications were detected by normal RT-PCR or with labelled primers (Genescan analysis) as described before.[20]

2.4. Immunophenotyping

Immunophenotyping was performed by fluorescence-activated cell sorting (FACS) analysis using a Becton Dickinson (BD, San Jose, CA, United States of America) FACSCalibur machine. Analysis was performed using Cellquest software (BD). White blood cells were isolated form BM or PB samples using lysing solution (Pharm lyse, BD) to eradicate red blood cells. After washing with PBS containing 0.1% human serum albumin (HSA), cells were resuspended in PBS containing 0.1% HSA, incubated with monoclonal antibodies (mAbs) for 15 min at room temperature and washed with PBS containing 0.1% HSA. Leukaemic blasts were characterised based on dim expression of peridinin chlorophyll protein (PerCp)-labelled CD45 (diluted 1:20, BD) and low SSC characteristics, in most cases combined with allophyocyanin (APC)-labelled CD34 (diluted 1:50, BD) expression. CD25 expression was determined using a PE-labelled mAb (diluted 1:10, Dako, Glostrup, Denmark). In order to identify the small blast compartment in normal BM, this population was identified by dim expression of CD45 PerCp, low SSC characteristics and positivity for CD34 APC and fluorescein isothiocyanate (FITC)-labelled CD13 (diluted 1:10, Dako). The percentage of CD25 positive blasts was defined as the expression above autofluorescence.

2.5. MRD detection

For MRD analysis, leukaemia associated phenotypes (LAPs) were established at diagnosis AML as described before.[5] MRD assessment was done after one and/or two and/or three courses of chemotherapy. BM cells from patients in CR and haematological recovery were treated with lysing solution to eradicate red blood cells. Next, cells were prepared as described in the previous paragraph and approximately 2×10^6 cells were incubated with the LAP defining antibody combinations in a volume of 50 μl. Previously defined cut-off levels for MRD cell frequency after different courses of chemotherapy were used for evaluating prognostic significance of MRD.[5]

Table 1 – Patient characteristics.

	Patients >60	Patients ⩽60*	Patients ⩽60 for survival analysis
No. of patients	44	72	65
Male/female	31/13	33/39	31/34
Age at diagnosis, Mean (range)	68 (61–82)	49 (18–60)	49 (18–60)
WBC count at diagnosis, 10^9/l, mean (range)	25 (0.8–388)	35 (0.4–322)	36 (8.7–322)
FAB classification, n (%)			
M0	1 (2.3)	1 (1.4)	1 (1.5)
M1	8 (18.2)	7 (9.7)	6 (9.2)
M2	7 (15.9)	17 (23.6)	17 (26.2)
M4	2 (4.5)	5 (6.9)	5 (7.7)
M5	3 (6.8)	14 (19.4)	13 (20)
M6	5 (11.4)	7 (9.7)	7 (10.8)
M7	1 (2.3)	0	0
RAEB	7 (15.9)	4 (5.6)	3 (4.6)
RAEB-t	1 (2.3)	5 (6.9)	5 (7.7)
AML with previous MDS	1 (2.3)	4 (5.6)	2 (3.1)
Not classified	7 (15.9)	8 (11.1)	6 (9.2)
Cytogenetic risk group, *n* (%)			
Favourable	2 (4.5)	8 (11.1)	8 (12.3)
Intermediate	33 (75)	44 (61.1)	38 (58.5)
Poor	6 (13,6)	9 (12.5)	9 (13.8)
No metaphases	3 (6.8)	11 (15.3)	10 (15.4)
FLT3-ITD, *n* (%)			
Present	7 (15.9)	23 (31.9)	19 (29.2)
Absent	37 (84.1)	49 (68.1)	46 (70.8)

RAEB = Refractory anaemia with excess blasts.
RAEB-t = Refractory anaemia with excess blasts in transformation.
*Including patients who received supporting care and patients who were treated elsewhere.

2.6. Statistical analysis

Statistical analysis of the data was done using the SPSS 14.0 software program. Cox regression was used to determine the correlation between the percentage of CD25 expression on leukaemic blasts and prognosis, to predict the relative risk of relapse with a 95% confidence interval (CI) and for univariate and multivariate analysis. Multivariate analysis was performed on a group of 55 patients ⩽60 all with known cytogenetics. Kaplan Meier analysis was used to evaluate the impact of cut-off values for CD25 expression and MRD cell frequency on clinical outcome. The log rank test was used to calculate the statistical significance. The Mann–Whitney *U* test for non-parametric samples was used to correlate CD25 expression with clinical and cellular features. *P*-values <0.05 were considered as statistically significant. Overall survival (OS) was defined as the period from the date of diagnosis until death and relapse free survival (RFS) was from the date of CR until relapse. Patients without a defined event were censored in statistical analysis at the time of last follow-up.

3. Results

3.1. CD25 positivity is predictive for clinical outcome in younger patients

In 65 chemotherapy-treated younger patients, CD25 expression when used as a continuous variable in Cox regression analysis was highly predictive for all survival parameters: OS ($p = 0.024$, $n = 65$) and RFS ($p = 0.00082$, $n = 56$). The median percentage of CD25 positive leukaemic blasts at diagnosis, was 17% (range: 0.3–91%). To define positivity, we chose a cut-off level of 10% CD25 positive blasts based upon the percentage of CD25 positive CD13+ CD34+ myeloid precursors from 12 healthy volunteers ⩽60 years (median: 6.0%, range: 1.9–9.9%). In the patient group ⩽60, a cut-off level of 10% CD25 positivity was highly significant in predicting OS and RFS ($p = 0.00045$ and $p = 0.0053$, respectively, as shown in Fig. 1a). Median OS was 10 months in the CD25 positive group ($n = 41$) versus >48 months ($n = 24$) in the CD25 negative group. Median RFS was 7 months ($n = 32$) and >47 months ($n = 24$), respectively. The relative risk of relapse was 3.9 (95% CI: 1.4–10.7, $p = 0.009$). When analysing subgroups of CD25 positivity (10–20% $n = 15$, 20–30% $n = 6$, 30–50% $n = 6$, 50–70% $n = 5$ and 70–100% $n = 9$) we found no significant difference between these groups in OS. For patients >60 years, Cox regression analysis showed no significant correlation with CD25 expression on leukaemic blasts at diagnosis, OS ($p = 0.29$, $n = 27$) and RFS ($p = 0.55$, $n = 20$). The percentage of CD25 positivity in this patient group was 37% (range: 0–89%), which is significantly higher as compared to patients ⩽60 years ($p = 0.08$). No cut-off could be established to significantly separate favourable and adverse prognostic patients in this group. We must therefore conclude that CD25 is not of prognostic value in our patient group >60 years, which is likely the result of the small number of patients available for

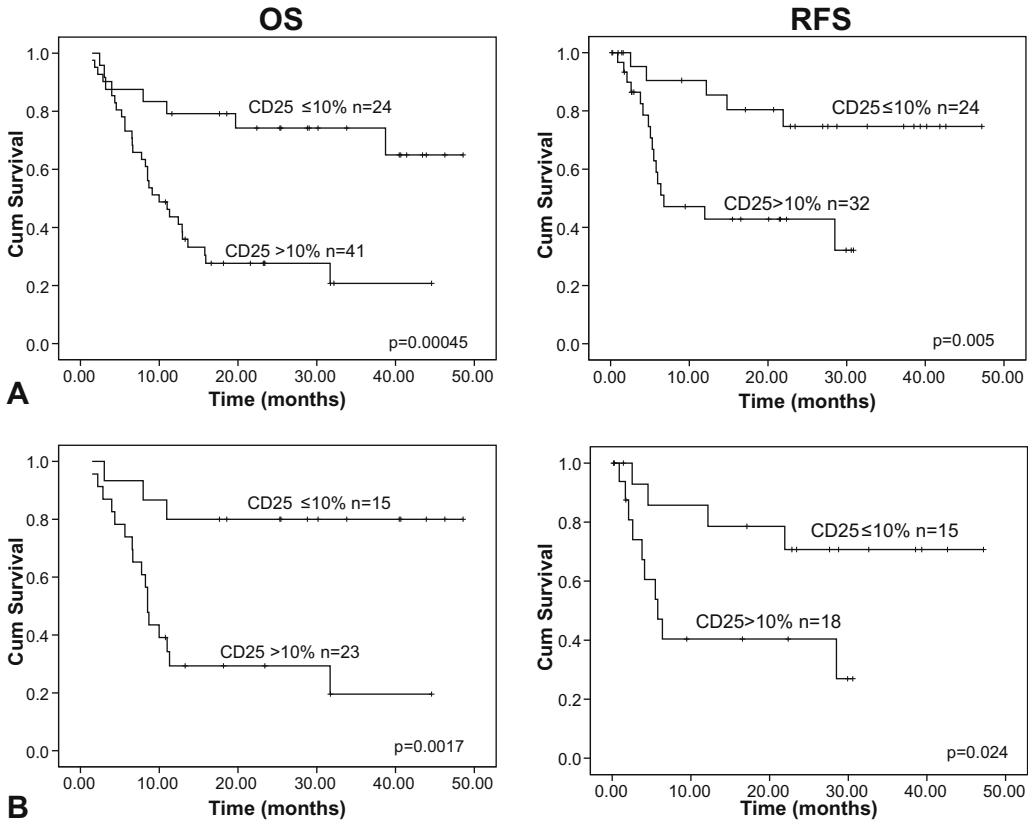

Fig. 1 – Kaplan-Meier plots of OS and RFS rates showing the prognostic value of CD25 in AML patients ⩽60 years in the whole group (a) and in the group with intermediate cytogenetics (b). Patients were assigned to high and low CD25 groups using a cut-off value of 10%.

survival analysis. From here on, further analysis has been performed only on the patient group ⩽60 years.

3.2. *CD25 is an independent prognostic factor*

To determine whether CD25 is of independent prognostic value in the younger patient group, univariate analysis was done with other well-known prognostic factors: cytogenetics, CR status, the presence of a FLT3-ITD and WBC count (Table 2). A cut-off value of 10% CD25 positivity was highly significant in predicting both OS and RFS. FLT3-ITD status and WBC had no prognostic impact in this analysis ($p = 0.12$ and $p = 0.44$ for OS, respectively), although a trend towards a shorter OS and RFS in FLT3-ITD patients compared to wild type could be distinguished. For RFS, CD25 positivity was the only and therefore independent poor prognostic marker for RFS. To analyse whether the percentage of CD25 positive leukaemic blasts is of independent prognostic value for OS, multivariate analysis was performed including all significant parameters from the univariate analysis. To include cytogenetics as a prognostic marker, multivariate analysis was performed only in patients with known cytogenetic risk ($n = 55$).

Table 2 – Prognostic factors for OS and RFS for patients ⩽60 years.

Variables	Univariate *P*-values		Multivariate *P*-values	
	OS	RFS	OS	RFS
%CD25 positivity[a]	0.0011	0.0093	0.0035[b]	0.0093
Cytogenetics	0.016	0.22	0.022[b]	NE
CR rate	0.0011	NR	0.218[b]	NE
FLT3-ITD	0.12	0.085	NE	NE
WBC	0.44	0.73	NE	NE

NR, not relevant.
NE, not evaluated due to lack of significance in univariate analysis.
a CD25 positivity defined with a cut-off value of 10%.
b For CD25 defined as a continuous variable these figures become 0.03, 0.07 and 0.02, respectively.

Table 2 shows that CD25 is an independent prognostic parameter for OS ($p = 0.0035$). Additionally, cytogenetics performed on 55 patients too was an independent prognostic factor for OS ($p = 0.022$).

3.3. Relationship between CD25 expression and other prognostic factors of AML

Because CD25 is associated with proliferation, we evaluated the relationship between CD25 and other parameters of blast proliferation: WBC count and FLT3-ITD status (Table 3). The percentage of CD25 positive blasts was found significantly higher in patients with a FLT3-ITD ($p = 0.004$). However, no significant correlation was found between the percentage of CD25 positive blasts and WBC count at diagnosis ($p = 0.60$). CD25 positivity was found to be significantly higher in refractory patients ($p = 0.021$, Table 3), which shows that CD25 is not only an independent factor for the duration of RFS (Fig. 1a), but also a predictor for achieving CR. No correlation was found between different FAB classes and the percentage of CD25 positivity, although in AML with MDS history ($n = 4$) the median expression was found to be high compared to other FAB classes (median: 40% versus 13%).

3.4. Prognostic relevance of CD25 in FLT3-ITD positive patients

The presence of a FLT3-ITD is associated with poor prognosis in AML.[9,21] In our patient group, only trends towards a shorter OS and RFS time in FLT3-ITD positive patients were found compared to wildtype patients ($p = 0.12$ and $p = 0.078$, respectively) which has been described previously for a small patient group.[22] When the two groups were considered separately, CD25 positivity (>10%) was found to be of significant prognostic impact for both OS and RFS in FLT3-ITD positive patients ($p = 0.023$ and $p = 0.047$, respectively) and in the group of FLT3 wildtype patients for OS ($p = 0.015$) and borderline significance for RFS ($p = 0.080$). In the FLT3-ITD positive group, patients with >10% CD25 positive blasts ($n = 11$) showed a median RFS of 6 months, while the group showing lower CD25 positivity ($n = 5$) had a median RFS of >38 months. For FLT3 wildtype patients, these figures were 28 months ($n = 21$) and >47 months ($n = 19$), respectively. Since the FLT3-ITD group for survival analysis consisted of only 19 patients, we used an additional set of 18 cryopreserved samples of FLT3-ITD positive ⩽60 years and assessed CD25 positivity in these. When considering all fresh and cryopreserved FLT3-ITD positive samples together, a cut-off of 10% CD25 positive blasts resulted in a loss of significance for OS (median: 9 months versus 16 months, $p = 0.23$), and RFS (median 6 months versus >38 months, $p = 0.20$). However, in this group, FLT3-ITD positive patients who were also CD25 positive (>10%) showed a significantly shorter OS (median 8 months versus 25 months, $p = 0.003$) and RFS (median 6 months versus >48 months, $p = 0.001$) as compared to CD25 low and FLT3 wildtype patients.

Table 3 – Correlation between CD25 expression and prognostic factors in AML.

	% CD25 expression Median (range) (*n*)	*P*-value
FLT3-ITD status		
Heterozygous	41 (1.9–85) (**23**)	0.004
Wt FLT3	12 (0.3–91) (**49**)	
WBC count		
$<5 \times 10^9$/l	14 (0.3–91) (**31**)	0.60
$\geqslant 5 \times 10^9$/l	19 (0.4–86) (**41**)	
CR status		
Reached	12 (0.3–91) (**56**)	0.02
Not reached	28 (11–86) (**9**)	

3.5. CD25 predicts prognosis within intermediate cytogenetics risk group

As additional prognostic markers are most valuable in the large intermediate risk group, the prognostic value of high CD25 expression was investigated in this group in specific. Again, CD25 positivity was associated with decreased median OS of 10 months ($n = 23$) versus >48 months ($n = 15$) in the CD25 negative group ($p = 0.0017$) as shown in Fig. 1b. For RFS, these figures were 6 months ($n = 18$) versus >47 months ($n = 15$, $p = 0.024$, Fig. 1b). The relative risk of relapse was 3.6 (95% CI: 1.1–11.5, $p = 0.03$). In addition, we investigated the prognostic impact of CD25 when combined with FLT3 status in this patient group. OS and RFS tended to be worse for patients with CD25 >10% than for patients with CD25 ⩽10% among FLT3 wildtype patients (not significant) and were even poorer for CD25 positive patients who also showed FLT3-ITD positivity. The latter group showed significantly shorter OS ($n = 9$, $p = 0.001$) and RFS ($n = 6$, $p = 0.00008$) as compared to the other patients.

3.6. CD25 expression correlates with MRD

Previously, we showed that MRD cell frequency after the first, second and third cycles of chemotherapy is a strong independent prognostic risk factor for survival.[5] Also in our current patient group ⩽60 years, MRD after the first and third cycles of chemotherapy is of prognostic value for OS and RFS. After one cycle of chemotherapy, MRD cell frequency above 0.1% showed a significantly worse OS ($p = 0.013$) and RFS ($p = 0.0089$) compared to MRD levels below cut-off. Median RFS was 6 months for patients with MRD cell frequency above cut-off level ($n = 16$), while for patients with MRD equal to or below 0.1% this was >47 months ($n = 23$, $p = 0.0089$). Because both the percentage of CD25 positive blasts at diagnosis and MRD detected after the first cycle of chemotherapy have prognostic impact, we hypothesised that the prognostic impact on survival of CD25 positivity is mediated by its direct correlation with MRD cell frequency. Indeed, after the first course of chemotherapy, more than 10% CD25 positive blasts was found to correlate with significantly higher MRD cell frequency (factor 6, $p = 0.040$, Fig. 2). After the second and third cycles of chemotherapy, despite the low number of patients (27 and 11, respectively), there were trends of correlation with RFS and OS, with respectively 1.3-fold and 3-fold higher MRD cell frequencies in the CD25 positive group.

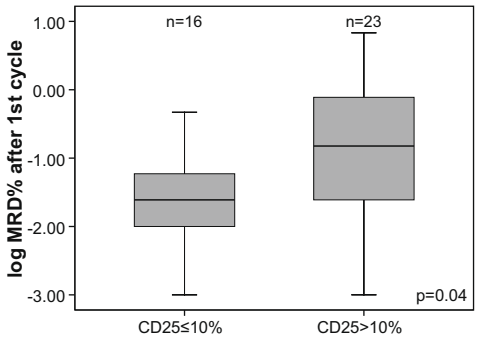

Fig. 2 – Correlation between CD25 expression at diagnosis and MRD cell frequency after the first cycle of chemotherapy. Patients were assigned to high and low CD25 groups using a cut-off value of 10%.

4. Discussion

AML treatment stratification for younger patients is currently based on cytogenetic characteristics. However, for fine-tuning prognostic risk of patients within the intermediate cytogenetic risk group, additional prognostic markers are required. Bearing in mind that most younger patients will enter CR, the most useful prognostic factors are those that predict relapse.[9] In this study, we retrospectively evaluated the impact of CD25 positive leukaemic blasts as a prognostic marker. The cut-off value for positivity of 10% CD25 expression on leukaemic blasts was selected based on the maximal expression on CD34 positive myeloid blasts in normal BM. Here we showed that in AML patients below the age of 60 CD25 positivity of leukaemic blasts at diagnosis is of prognostic value with regard to OS and RFS. However, most probably due to the low number of patients, no such cut-off level could be detected for patients over 60 years. Multivariate analysis showed the percentage of CD25 positive blasts, together with cytogenetics, to be an independent predictor for OS and the only prognostic factor for RFS in the younger patient group.

Although the karyotype at diagnosis provides the most important prognostic information, only a minority of patients can be classified into the adverse (10%) or favourable risk category (23%).[19] The recent focus in AML is therefore on prognostic factors in the intermediate cytogenetic risk group. These include the good prognostic mutations in NPM1 and CEBPα,[23] microRNA expression[24] and the expression of apoptosis-related genes.[25] Of utmost importance in the current study is the strong prognostic impact of CD25 expression in the group with intermediate prognosis (Fig. 1b) which includes the majority of AML patients.

Similarly, using FLT3-ITD as genotypic-defined poor prognostic parameter,[21] CD25 predicted poor response in the FLT3 wildtype group which makes up the majority (76%) of patients.[26] Among FLT3-ITD patients, OS and RFS tended to be worse for CD25 positive patients. Since most FLT3-ITD patients are part of the intermediate cytogenetic risk group, we expected the same impact for CD25 among FLT3-ITD patients in this patient group. Indeed, by combining FLT3-ITD positivity with CD25 levels >10% patients with the shortest OS and RFS could be identified.

High CD25 had a negative impact on both achieving CR and subsequent time to relapse. These are not necessarily independent factors: we showed that even when achieving CR (<5% blasts in the BM) the frequency of residual cells is proportional to CD25 expression. As we and others have shown in turn that this frequency of residual cells has strong independent prognostic impact for time to relapse and OS,[5–8] the present results show that CD25 expression is directly related to quality of CR. To explain the observed impact on RFS there is no need to postulate additional factors. The CD25-associated mechanism involved in affecting RFS is thereby likely the same as those affecting the achievement of CR.

Despite previous research regarding CD25, the biological significance of CD25 expression on AML blasts remains unclear. The overall function of CD25 on T-cells is to increase the binding affinity of IL-2 to its receptor for activation and proliferation. Whether this function also applies to AML, however, remains unclear as heterogeneous effects have been described: varying from reduction, to no effect to an increase in proliferation after IL-2 stimulation.[27–30]

Besides its role in forming the high affinity IL-2 receptor, CD25 can be enzymatically cleaved and shed from the surface of CD25 positive cells as soluble IL-2Rα (sIL-2Rα), proportional to its cell surface expression.[31] High plasma levels of sIL-2Rα were detected in a number of pathological states, including AML,[32] which was associated with an adverse prognosis. The mechanism behind the adverse prognosis of CD25 positive AML patients could be that sIL-2Rα, together with surface CD25 expression, leads to competition for IL-2 and suppression of the antitumour T-cell and NK-cell activity in AML.[17] IL-2 administration might therefore play a role in activating the immune system. We found that the levels of cell surface CD25 were significantly higher in older patients compared to patients ⩽60 years. This might explain the lack of effect of IL-2 immunotherapy in older patients with AML, because IL-2 administration in younger patients does seem to have a positive effect.[33]

We recently found that CD25 is positively correlated with the progenitor cell surface marker CD34, which suggest that CD25 expression is highest on more primitive AML subtypes (not published). This raises the possibility that the correlation of CD25 with MRD and survival parameters is not mediated by the function of CD25, but more likely reflects the degree of primitivity/differentiation of the blasts cells, which is in turn associated with all kinds of therapy resistant mechanisms. In agreement with that it is important to acknowledge that, similar to CD25 expression, expression and function of the drug extrusion pump P-glycoprotein and the expression of apoptosis resistance proteins are related to MRD cell frequency as well as survival.[2,3] The latter result may suggest that at diagnosis, activation of multiple resistance mechanisms directly affecting therapy resistance, or indirectly via immune escape mechanisms.[34] It would therefore be of great interest to prospectively study how such resistance mechanisms are co-upregulated in diagnosis AML. Experiments to substantiate this are underway.

Conflict of interest statement

None declared.

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