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P-glycoprotein and multidrug resistance associated protein-1 activity in 132 acute myeloid leukemias according to FAB subtypes and cytogenetic risk groups

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A B S T R A C T

Background and Objectives. We studied the function of both P-glycoprotein (Pgp) and multidrug resistance associated protein-1 (MRP1) to identify subgroups of patients who could benefit from Pgp reversion, and to clarify the expression and function of these proteins in different FAB subtypes and cytogenetic risk groups.

Design and Methods. We examined Pgp and MRP1 expression and function in 132 adults with *de novo* acute myeloid leukemia *(*AML). We correlated our findings with the FAB subtypes and cytogenetic risk groups, and clinical data of our patients.

Results. Patients with good risk cytogenetics have low expression and activity of Pgp and MRP1 except patients with inv(16) who have a higher activity of MRP1 than do patients with t(8;21) and t(15;17) (p=0.05). All other AML patients, except those with M5, have high expression and activity of Pgp. In contrast, patients with M5 AML have a high expression, but low activity of Pgp. In this subgroup, patients with M5 AML and *MLL* gene rearrangement did not express active Pgp. Others patients with M5 AML did not have functional Pgp. Patients with monosomy 7, *11q2.3* gene rearrangement and complex cytogenetics have higher activity of MRP1 than those with other cytogenetic findings (p=0.03).

Interpretation and Conclusions. The resistance mechanism in M5 was not mediated by Pgp. In contrast, MRP1 may play a role in patients who have a *11q2.3* gene rearrangement, or in M4E with inv(16). Thus trials that modulate Pgp are likely to achieve limited success in cases of AML with low activity of Pgp, i.e., M5, and AML with good risk cytogenetics.

Key words: Pgp, MRP1, cytogenetic, FAB subtypes, acute monocytic leukemia, acute myeloblastic leukemia.

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Resistance to chemotherapy is one of the major obstacles to effective treatment in acute myeloid leukemia (AML).[1] Despite improvements in these thirty last years achieved by the use of combinations of cytarabine and intercalating agents, the overall prognosis remains poor.[2] One of the best characterized resistance mechanism in AML is drug extrusion mediated by the ABCB1 protein (MDR-1/Pgp, P-glycoprotein), which has been shown to be associated with a poor outcome.[3,4] Therefore, several randomized trials of Pgp modulation in AML and myelodysplastic syndromes have been performed.[5-9] Discrepant results emerged from these studies. The data suggested that adult patients with *de novo* AML are less suited for multiple drug resistance (MDR) reversion than are relapsed/refractory AML patients and high-risk myelodysplastic syndrome patients. In the study by Solary *et al.* quinine did not improve the survival of patients with *de novo* AML. Nevertheless, the study demonstrated that the response to the induction regimen was decreased in patients whose blast cells demonstrated rhodamine123 efflux (Pgp activity), a negative effect that was corrected by modulator administration. Difficulties in defining which method is most appropriate for determining the multidrug resistance (MDR) status of a patient's blast cells have complicated the identification of those patients who could benefit from MDR-reversing strategies. In addition, Broxterman *et al.* showed that Pgp function in AML cells did not correspond to *in vitro* cytotoxicity.[10] Several consensus recommendations have been reported in an attempt to decrease variability in the measurement of MDR factors.[11,12] In addition, several studies recommended using functional

assays to identify patients who could benefit from MDR reversion and to assess MDR1.[5,7,12,13,14]

Promyelocytic leukemia (APL/M3) has been reported in the literature as expressing low levels of Pgp with a low activity, providing the biological basis for the high sensitivity of this leukemia subtype to anthracyclines.[15] Moreover, APL is often CD34 negative and Pgp function has been strongly correlated with CD34 positivity.[16] The other FAB subtypes and cytogenetic risk groups of AML are not clearly associated with a particular phenotype of expression or function of Pgp; however, in a small subset of cases, monocytic leukemia (M5) seems to correlate negatively with Pgp function, without a better prognosis.[3,17,18] Analysis of Pgp function according to FAB and cytogenetic subtypes would be able to identify the subgroups of patients who could benefit from Pgp modulation more precisely.

However, in several studies, discrepant cases were reported, with increased efflux and no significant MDR1 expression.[14] This suggests that alternative proteins, such as the ABCC1 protein (MRP1)[19] or the lung resistance protein (LRP),[20] may contribute to the MDR phenotype in AML.

We, therefore, retrospectively studied the level of Pgp expression and its activity, the level of MRP1 expression and function and the level of LRP expression at diagnosis, in a cohort of 132 AML patients, stratified according to FAB subtype and karyotype. We also report the patients' clinical outcome.

Design and Methods

Patients

One hundred and thirty-two patients were studied for Pgp, MRP1 and LRP expression level and for JC-1 (Pgp function) and calcein-AM (MRP1 function). The diagnosis of AML was based on cytological and cytochemical examination of bone marrow smears according to the French-American-British (FAB) criteria.[21] Immunophenotyping was performed using a FACSORT flow cytometer (Becton Dickinson, France). CD34 positivity was defined as expression of this molecule on more than 20% of the blasts. All samples were tested at diagnosis.

For each patient several clinical and biological characteristics were analyzed (age, white blood cell (WBC) count, serum lactate desyhdrogenase (LDH) level, CD34 expression and karyotype). Unfavorable karyotypes were defined as abnormalities of chromosome 5 or 7, or abnormalities of the 11q2.3 band or complex abnormalities. Inversion in chromosome 16 (inv 16) or t(8;21) indicated good prognosis, and the other karyotypes, including normal ones, indicated an intermediate prognosis. Patients with t(9;22) were not included in this study. Patients included in our analysis were treated intensively with one of the EORTC protocols or with comparable therapies.

Flow cytometric detection of Pgp expression and function

Pgp expression was measured by labeling fresh viable cells with the UIC2 monoclonal antibodies (MoAbs) and phycoerythrin (PE)-labeled second antibody as described before.[22] As previously reported, the same results were found with MRK16 antibody (r=0.9, 60 patients). Therefore, only the results with UIC2 are presented. Pgp expression was established on blast cells selected by CD34 antibody (HPCA2 clone, Becton Dickinson, France; two color assay) or other markers (for example CD33/CD7, CD33/CD2, CD33/CD19 or CD33/CD22 by three-color assay) whenever possible, or by physical characteristics only if the blast cells did not express characteristic markers. Fluorescence was analyzed on a FACSORT flow cytometer (Becton Dickinson, France). UIC2 staining was measured, as recommended, using the Kolmogorov-Smirnov (KS) statistic to compare UIC2-stained cells with the controls; the KS statistic measures the difference between two distribution functions and generates a D value ranging between 0 and 1.0, with higher values indicating a greater difference between the distribution functions.[22] As explained in our previous works and elsewhere, Pgp expression was correlated with clinical data using the D value as a continuous variable.

JC-1 is a carbocyanine liquid crystal forming probe. This cationic dye was initially used for analysis of mitochondrial potential and is a reliable probe for analyzing changes occurring very early in apoptosis. JC-1 is also a fluorescent molecule, recently described as a probe for Pgp, and more sensitive than rhodamine 123.[4,23] In order to stain these cells, they were washed twice and re-suspended in PBS containing 0.1 mM JC-1 monomer at a concentration of 5×10^5 cells/mL and incubated at 37°C (pH=7.4) for 15', in a CO_2 incubator, with or without modulator (CsA [2 mmol/L]) to assess Pgp function. Cells were washed twice in cold PBS and samples were analyzed. JC-1 fluorescence was measured on the FL1 channel by detection of the fluorescence of the dye monomer. Pgp function was established using blast cells selected by CD34 antibody (FL3 channel; HPCA2 clone, Becton & Dickinson, Le Pont de Claix, France), or by physical characteristics only if blast cells did not express characteristic markers. The intensity of JC-1 uptake was measured using the KS statistic D, calculating the difference in fluorescence intensity of the blasts in the presence and absence of CsA; a higher D value indicates a wider difference between Pgp function in the two cases, and thus a more resistant group of cells. As explained in our

previous works and elsewhere, Pgp function was correlated with clinical data using the D value as a continuous variable.

Level of both MRP1 expression and function and LRP expression

The expression of MRP1 and LRP was measured by labeling fresh viable cells with the MRPm6 and LRP56 monoclonal antibodies, respectively and then with a PE-labeled second antibody as described elsewhere.[22] The expression of these proteins was established as reported above.

In a previous study, we showed that calcein-AM uptake ± probenecid provided a functional test for MRP1 in leukemic cells.[24] Cells were incubated with 0.1 μmol/L of calcein-AM for 15 minutes at 37°C (pH=7.4), in a CO_2 incubator, in RPMI medium with or without probenecid [2 mmol/L], a modulator of MRP1. Cells were then washed twice in cold PBS and samples were analyzed with a FACSORT flow cytometer. The function of MDR proteins was established in blast cells selected as above. The intensity of calcein was measured using the KS statistic D, calculating the difference in fluorescence intensity of the blasts in the presence and absence of probenecid; a higher D value indicates a wider difference between MRP1 function in the two cases, and thus a more resistant group of cells.

Statistical analysis

The association between variables was analyzed using Fisher's exact test for categorical variables and by the Mann Whitney U or Kruskal Wallis test for continuous variables. Survival curves were plotted according to the method of Kaplan-Meier, and compared by the log rank test. Overall survival (OS) and disease-free survival (DFS) were analyzed. DFS was defined as survival without relapse in the group of patients who achieved complete remission (CR) after induction therapy. The odds ratio (OR), with 95% confidence intervals (CI), is given for the principal comparisons of the major end-points (CR, DFS and OS). The median follow-up of patients who remained alive was 4 years.

Results

Pgp expression and Pgp function in the 132 AML patients

The expression of Pgp was not a prognostic factor, or only a weak one, for achievement of CR (p = NS), duration of DFS (OR=2.0 [95% CI 0.9-5.7], p = 0.06) and duration of OS (OR=1.9 [95% CI 1.01-4.9], p = 0.05). In contrast, Pgp function was a strong prognostic factor for achievement of CR (0.48±0.29 in patients with no CR versus 0.35±0.20 in patients with CR, p = 0.03), duration of DFS (OR= 2.9 [95% CI 0.9-8.7], p = 0.05) and duration of OS (OR= 2.68 [95% CI 1.28-5.63], p = 0.009). These correlations were increased when patients with M5 were removed from the analysis (OR= 3.5 [95% CI 1.3-6.2], p = 0.03 for DFS, and OR= 4.07 [95% CI 1.8-9.1], p = 0.0007 for OS). Indeed, patients with M5 did not have functional Pgp (*see below*). Therefore both expression and function of Pgp predict outcome, but Pgp function was a better predictor than Pgp expression. We, thereafore, specifically analyzed Pgp function according to FAB subtypes and cytogenetic risk groups.

Pgp function and cytogenetic groups

Among the 132 patients, 122 had information from a cytogenetic analysis (Table 1). The DFS and OS of the patients with unfavorable, intermediate and good risk cytogenetics are illustrated in Figure 1. AML patients with good risk cytogenetics had lower Pgp function than did those with intermediate and poor risk cytogenetics (0.08±0.04 versus 0.40±0.30 versus 0.50±0.40, p = 0.04). The intermediate risk group did not have lower Pgp function than the poor risk cytogenetic group. In the patients with poor risk cytogenetics, the *11q2.3* gene rearrangement was the sole chromosome abnormality to be associated with non-functional Pgp (0.10±0.04 versus 0.59±0.45, p = 0.04). Therefore, when patients with 11q2.3 gene rearrangement were removed from the analysis, those with poor risk cytogenetics had higher Pgp function than those with intermediate risk cytogenetics (0.59±0.45 versus 0.40±0.30, p = 0.03).

Pgp function and FAB subtypes

There was also heterogeneity of Pgp expression and function among patients with different FAB subtypes and in different cytogenetic risk groups (Table 2). AML can be subdivided into 4 groups according to Pgp expression and function. The DFS and OS of patients in these groups are represented Figure 2. A first group had both a low level of Pgp expression and low Pgp function: M2 with t(8;21)(q22;q22) (7 patients, 0.1±0.02 and 0.08±0.01, respectively), M4 with inv(16)(p13q22) (6 patients, 0.08±0.02 and 0.09±0.07, respectively), and M3 (6 patients, 0.09±0.05 and 0.1±0.04, respectively); this group of patients had good risk cytogenetics. A second group had both a high level of Pgp expression and high Pgp function: M0 (2 patients), M1 (18 patients, 0.28±0.05 and 0.51±0.08, respectively), M2 without t(8;21) (55 patients, 0.31±0.04 and 0.52±0.04, respectively), M6 (4 patients, 0.3±0.15 and 0.46±0.15, respectively) and M7 (2 patients). All these patients had either poor or intermediate risk cytogenetics. There was a statistical difference between these two groups (0.54±0.2 versus

Table 1. Pgp expression and function, MRP1 expression and function, and LRP expression among cytogenetic risk groups.

Cytogenetic risk group and karyotype	*N*	*Pgp*		*MRP1*		*LRP*
		Expression	*Function*	*Expression*	*Function*	*Expression*
		Mean value ± SEM		*Mean value ± SEM*		*Mean value ± SEM*
Good	19	0.08±0.02	0.08±0.04	0.20±0.09	0.19±0.12	0.25±0.17
t(15;17)	6	0.09±0.05	0.10±0.04	0.15±0.10	0.15±0.11	0.20±0.12
Inv(16)	6	0.08±0.02	0.09±0.07	0.31±0.08	0.25±0.08	0.22±0.11
t(8;21)	7	0.10±0.02	0.08±0.01	0.15±0.10	0.18±0.14	0.31±0.21
Intermediate	75	0.29±0.12	0.40±0.30	0.27±0.20	0.25±0.18	0.23±0.15
Normal	53	0.30±0.11	0.43±0.28	0.29±0.24	0.27±0.12	0.26±0.19
+8	9	0.21±0.19	0.40±0.38	0.24±0.10	0.21±0.14	0.21±0.12
Others	13	0.36±0.09	0.35±0.20	0.23±0.17	0.27±0.21	0.19±0.16
Poor	28	0.38±0.31	0.50±0.40	0.34±0.18	0.29±0.18	0.35±0.15
-5/del(5)	4	0.35±0.25	0.49±0.50	0.27±0.14	0.24±0.12	0.27±0.16
-7/del(7)	10	0.38±0.39	0.60±0.30	0.39±0.23	0.36±0.26	0.31±0.12
11q2.3	6	0.32±0.10	0.10±0.04	0.28±0.12	0.32±0.11	0.27±0.17
Complex	8	0.40±0.24	0.70±0.20	0.37±0.12	0.38±0.25	0.38±0.17

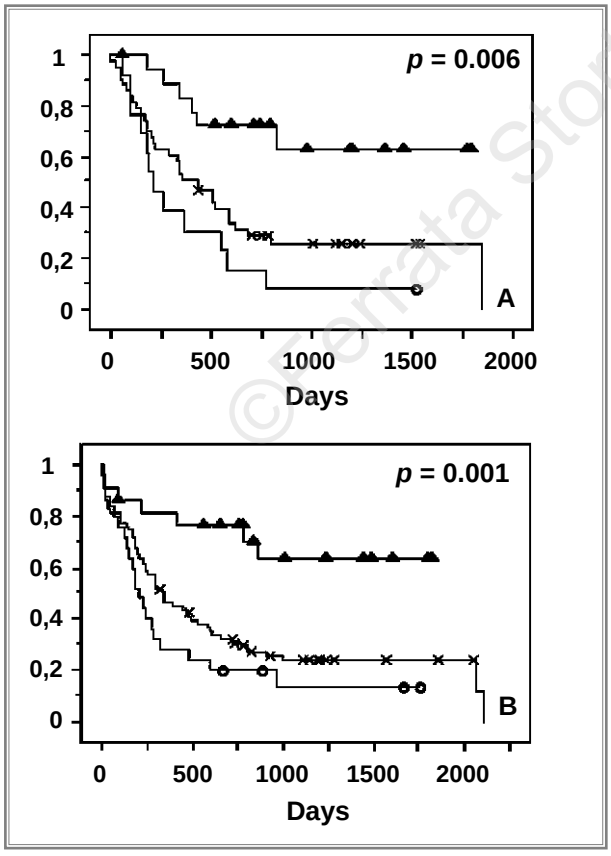

Figure 1. DFS (A) and OS (B) of 122 AML patients according to cytogenetic risk group. ▲ good risk; × intermediate risk; ○ poor risk.

0.42±0.2 respectively, $p = 0.03$). A third group had a high level of Pgp expression, but an intermediate level of Pgp function: M4 without inv(16). All these patients also had either poor or intermediate risk cytogenetics (18 patients, 0.38±0.05 and 0.30±0.06, respectively). The last group was formed of patients with a high level of Pgp expression, but low activity of Pgp: M5 (14 patients, 0.30±0.05 and 0.16±0.06, respectively). In this group, patients with M5 with *11q2.3* gene rearrangement (poor risk cytogenetics) did not express active Pgp in contrast to patients in the other poor risk cytogenetic groups. However, other patients with M5 AML also did not have functional Pgp and two further patients with *11q2.3* gene rearrangement (one M2 and one M4) did not express Pgp. The clinical and biological characteristics of these four groups are shown in Table 3.

Patients with acute myelomonocytic leukemia without inv(16) had an intermediate level of Pgp activity compared to the levels in M0, M1, M2, M6, and M7 which showed high Pgp activity and in M2 (t(8;21)) M4E, M3, M5 which showed low Pgp activity (0.38±0.05 versus 0.51±0.15 versus 0.14±0.09, respectively, $p = 0.03$) (Table 2). In this subtype, monocytes and their precursors, selected by CD14 antibody or physical characteristics, had high Pgp expression but the activity of this protein was weak, as in M5 (Table 4). The myeloblastic component of M4 had, as in M1 and M2, a higher Pgp activity than did the monocytic component (0.60±0.10 versus 0.10±0.07, $p = 0.04$) (Table 4).

Table 2. Pgp expression and function and CD34 expression among FAB subtypes.

FAB Categories	Patients n (%)	Pgp Expression mean value ± SEM*	Pgp Function mean value ± SEM*	CD34 positivity (%)
M0	2 (1)	–	0.40±0.36	100
M1	18 (13)	0.28±0.05	0.51±0.08	66
M2	55 (42)	0.31±0.04	0.52±0.04	83
M2, t(8;21)	7 (5)	0.1±0.02	0.08±0.01	71
M3	6 (4)	0.09±0.05	0.10±0.04	0
M4	18 (14)	0.30±0.06	0.38±0.05	58
M4 inv(16)	6 (4)	0.08±0.02	0.09±0.07	33
M5	14 (10)	0.30±0.05	0.16±0.06	21
M6	4 (3)	0.30±0.15	0.46±0.15	75
M7	2 (1)	–	0.79±0.03	100

SEM: standard error of mean value.

MRP1 expression and function and LRP expression in FAB subtypes and cytogenetic risk groups

There was not significant heterogeneity of LRP or MRP1 expression and MRP1 function among different FAB subtypes (Table 5). In contrast, there was a difference for both MRP1 expression and function between patients with intermediate and good risk cytogenetics (0.27±0.20 versus 0.20±0.09, $p = 0.04$ for expression; 0.25±018 versus 0.19±012, $p = 0.05$ for function, respectively) and between poor and good risk cytogenetics (0.34±0.18 versus 0.20±0.09, $p = 0.03$ for expression; 0.29±0.18 versus 0.19±012, $p = 0.04$ for function) (Table 1). Among the good cases with risk cytogenetics, (those with Inv(16) had higher activity of MRP1 than those with t(8;21) or t(15;17) (0.25±0.08 versus 0.16±0.12, $p = 0.05$). Monosomy 7, *11q2.3* gene rearrangement and complex cytogenetics were associated with MRP1 higher activity than were other cytogenetic groups (0.34±0.19 versus 0.22±0.18, $p = 0.03$). There was not a significant heterogeneity of LRP expression between cytogenetic groups.

Discussion

It is now well established that overexpression and especially overactivity of Pgp is associated with an unfavorable prognosis.[1,3,4,18,22,24] Nevertheless, the randomized studies of Pgp modulators in AML gave contradictory results.[5,7,8,9] Several studies argued strongly for using functional assays to identify patients who could benefit from MDR reversion.[5,13,14] Therefore, we analyzed the Pgp, MRP1, and LRP status according to FAB subtypes and cytogenetic risk groups in adult

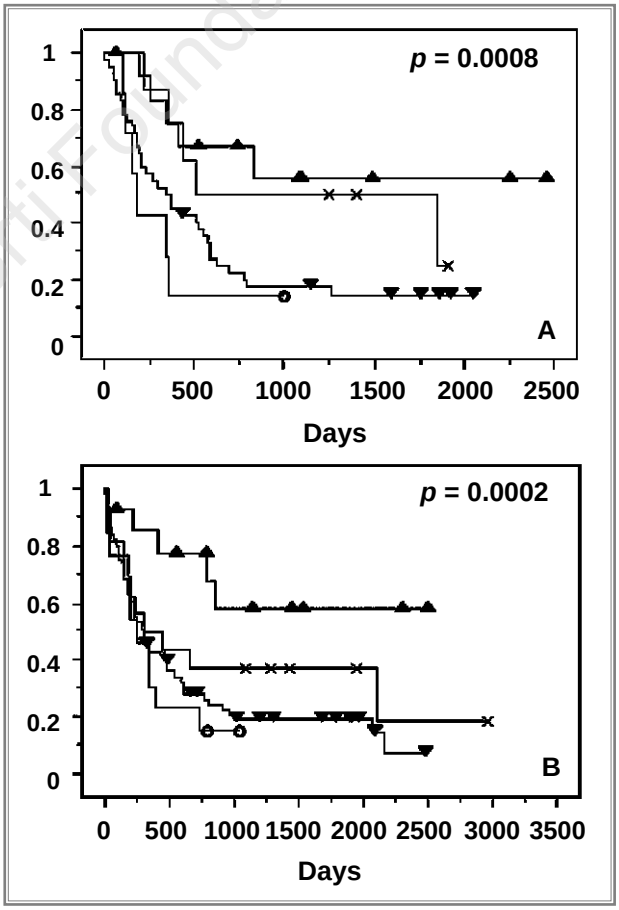

Figure 2. DFS (A) and OS (B) of AML patients, in four groups, according to both Pgp expression and activity (1) ▲ low level of both Pgp expression and activity (M2 with t(8;21), M4 with inv(16) and M3); (2) ×: high level of Pgp, but intermediate Pgp activity (M4 without inv(16); (3) ▼ high level of both Pgp expression and function (M0, M1, M2 without t(8;21), M6 and M7); (4) ○: high level of Pgp expression and very low level of Pgp function (M5).

Table 3. Comparison of clinical and biological characteristics between four groups according to both expression and function of Pgp: (1) M2 with t(8;21), M3, and M4 with inv(16) with weak expression and function of Pgp ; (2) M0,M1,M2 without t(8;21), M6, and M7 with both high expression and activity of Pgp ; (3) M4 without inv(16) with high expression of Pgp and intermediate activity of Pgp ; and (4) M5 with high expression of Pgp, but low activity of Pgp.

	M2, M4* and M3 n=19*	*M0, M1, M2, M6 and M7§ n=81*	*M4** n=18*	*M5 n=14*	*p*
Age (years)	42±11	56±18	60±14	52±19	0.02
WBC (G/L)	40±64	49±84	78±75	68±64	0.01
LDH (U/L)	1576±1472	1837±2062	1636±1500	2568±1951	NS
CD34 expression (% of patients)	92%	78%	37%	23%	<0.0001
Karyotype	19 patients	74 patients	16 patients	13 patients	NS
Good (n, %)	19 (100%)	0	0%	0	
Intermediate (n, %)	0	54 (73%)	14 (87%)	8 (64%)	
Normal		35	11	7	
+8		7	2	0	
Other		12	0	1	
					NS§
Unfavorable (n, %)	0	21 (27%)	2 (13%)	5 (46%)	
-5		4	0	0	
-7		10	0	0	
Complex		6	1	1	
11q2.3		1	1	4	
WHO performance status 0,1 versus 2,3,4	85%/15%	73%/27%	86%/14%	33%/67%	0.009
Death in induction	5%	13%	11%	28%	NS
Failure of induction treatment	0%	27%	32%	12%	0.02
Complete remission after induction	95%	60%	57%	54%	0.02

**Including only M2 with t(8;21) and M4 with inv(16); §did not include M2 with t(8;21); **did not include M4 with inv(16) §p value did not include M2 with t(8;21), M4 with inv(16) and M3.*

Table 4. Expression and function of Pgp, MRP1 and LRP in acute myelo-monocytic leukemia (M4 without inv(16)), both in monocytic and myeloblastic components.

M4 (18 patients)	*Pgp expression*	*Pgp function*	*MRP1 expression*	*MRP1 function*	*LRP expression*
Monocytic component	0.27±0.02	0.10±0.07	0.32±0.09	0.29±0.10	0.31±0.20
Myeloblastic component	0.34±0.05	0.60±0.10	0.25±0.10	0.24±0.12	0.36±0.14
p value	NS	0.04	NS	NS	NS

patients with AML to identify precisely the groups of patients who overexpressed functional proteins and who could benefit from Pgp modulation. It is now well established that patients with M3 present a very low frequency of Pgp expression and activity.[15] In our study, M3 patients also did not express functional MRP1. However, it is not clear whether other FAB subtypes or cytogenetic groups have particular increases or decreases in expression or function of these proteins. Most studies analyzed protein expression, but not Pgp function.[3,17,18]

In our study, patients with M5 expressed MDR1 but the functional level of the protein was very low. As expected, the other AML subtypes with high Pgp expression (M0, M1, M2 without t(8;21), M4 without inv(16), M6, and M7) had a high activity of Pgp and a

Table 5. MRP1 expression and function, and LRP expression among FAB subtypes.

FAB		*MRP1*		*LRP*
Categories	*patients n (%)*	*Expression Mean value ± SEM*	*Function Mean value ± SEM*	*Mean value ± SEM*
M0	2 (1)	–	–	
M1	18 (13)	0.29±0.07	0.30±0.09	0.30±0.12
M2	55 (42)	0.35±0.09	0.31±0.05	0.40±0.25
M2, t(8;21)	7 (5)	0.15±0.10	0.18±0.14	0.31±0.21
M3	6 (4)	0.15±0.10	0.15±0.11	0.20±0.12
M4	18 (14)	0.28±0.09	0.27±0.12	0.33±0.14
M4 inv(16)	6 (4)	0.31±0.08	0.25±0.08	0.22±0.11
M5	14 (10)	0.25±0.06	0.20±0.10	0.29±0.20
M6	4 (3)	0.15±0.11	0.21±0.13	0.37±0.25
M7	2 (1)	–	–	

poor outcome, except those with M4 without inv(16) who had intermediate Pgp activity. The other types of AML, M2 with t(8;21), and M4 with inv(16), displayed weak expression and activity of Pgp and had a good prognosis. A recent study demonstrated that MDR1 promoter is a target for AML1/ETO transcriptional repression.[25] Furthermore, in our study, we found lower expression of CD34 in M5 patients than in patients with other AML subtypes. As previously described, Pgp expression without functional drug efflux correlates with a lower expression of CD34 surface marker.[16] Therefore, patients with M5, which has inactive Pgp, should also have a better prognosis than that of patients with other AML subtypes. However, in our study, their prognosis was equivalent to that of patients with other FAB subtypes. Thus, other mechanisms of chemoresistance distinct from Pgp may explain the prognosis of patients with AML M5. Recently, a study by Pallis *et al.* indicated an efflux-independent role for Pgp as an anti-apoptotic molecule.[26] LRP was found to be overexpressed in the M5 FAB subtype of leukemia, in a group of young AML patients expressing MDR1 less frequently, but the exact role of this protein in chemoresistance is still controversial and, in recent reports, it has not been a significant predictor of outcome.[3,20] In our study, LRP was not overexpressed in this subtype of AML. Other trans-membrane proteins, such as MRP1, may play a role in drug resistance in AML.[22] The M5 subtype has been reported to be frequently associated with *MLL/11q2.3* gene rearrangement.[20-22] In our study, patients with this rearrangement did not express functional Pgp, and two patients with *11q2.3* gene rearrangement who did not have AML M5 also did not express functional Pgp. In contrast, these patients had a high value of MRP1 activity; this protein could play a role in chemoresistance in AML with *11q23* gene rearrangement. In the group of patients with good risk cytogenetics those with inv(16) had a higher activity of MRP1 than those with t(8;21) and t(15;17). In recent studies, adult AML patients with inv(16)[27] were found to have a shorter duration of DFS than adult patients with t(8;21)[28] and APL patients. Thus MRP1 could also play a role in chemoresistance in this subgroup of AML.

In conclusion, (i) resistance mechanisms in M5, and to a lesser extent in M4 without inv(16), were not mediated by Pgp; (ii) MRP1 may play a role in some of these patients who have a 11q2.3 gene rearrangement. MRP1 may also play a role in AML with inv(16), and in those with poor risk cytogenetics, in co-operation with Pgp; (iii) trials that modulate Pgp are likely to achieve limited success in FAB subtypes with low Pgp function: M5 and M4, M2 with t(8;21) or M4E with inv(16). This should encourage stratification of patients enrolled in MDR inhibitor trials according to Pgp function, or at least according to FAB groups and/or cytogenetic risk groups with high Pgp function.

Contributions. OL, SZ, J-YP, A-MF, ZB, DC, and J-PM, analyzed and interpreted the data; OL wrote the paper. The authors reported no conflict of interest.

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