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Brief communication

Fludarabine-based induction therapy does not overcome the negative effect of ABCG2 (BCRP) over-expression in adult acute myeloid leukemia patients

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ABSTRACT

Over-expression of multidrug resistance (MDR) proteins PGP and BCRP has a negative prognostic impact in acute myeloid leukemia (AML) patients. Inclusion of fludarabine in induction chemotherapy increases remission rate in PGP over-expressing cases. We investigated the role of BCRP in 138 adult AML patients receiving induction therapy with fludarabine. None of the MDR-related proteins influenced complete remission attainment. Conversely, high levels of BCRP significantly affected disease-free survival, as higher relapse rates (48.5% vs 28.5%) and earlier relapse occurred in BCRP+ patients. Also overall survival was affected by BCRP positivity, and survival significantly worsened in case of concomitant PGP and BCRP over-expression.

1. Introduction

Resistance of the neoplastic cell to chemotherapy represents a major obstacle towards the cure of acute myeloid leukemia (AML) patients. Over-expression of various multidrug resistance (MDR) proteins is associated with clinical resistance to chemotherapy and, consequently, treatment failure. P-glycoprotein (PGP), an ATP-dependent membrane transporter encoded by the ABCB1 gene, has proved to be associated with lower complete remission (CR) rates and shorter survival in different studies on de novo or secondary AML [1,2]. Other MDR-related proteins, such as MRP and LRP, can be abnormally expressed in AML blasts, but their role in the clinical setting is less defined. More recently, a new ATP-binding cassette protein, the breast cancer resistance protein (BCRP, or ABCG2), has been identified [3]. In vitro, BCRP confers resistance to many different compounds and plays an important role in affecting disposition of various chemotherapeutic agents, including mitoxantrone, daunorubicin, doxorubicin and topotecan [4]. After discordant results in the past years, there is increasing evidence that BCRP over-expression is associated with a worse prognosis in AML patients [5–7].

The inclusion of purine analogue fludarabine in induction chemotherapy for AML has achieved remarkable CR rates, proving to be highly active also in the subset of PGP over-expressing cases [8,9]. No specific data are available about fludarabine effect on the outcome of AML patients with BCRP over-expression.

In the present paper we have evaluated BCRP expression in 138 cases of AML treated with a fludarabine-based induction therapy, to evaluate the possible impact of BCRP on response to therapy and its ability to identify patients with poor prognosis, that may take advantage of a more intensive post-remission treatment.

2. Materials and methods

One hundred and thirty-eight consecutive patients that were diagnosed with AML between January 2003 and April 2008 and received induction chemotherapy with a fludarabine-based protocol at the Division of Hematology of Udine were included in this study. Clinical characteristics of the study population are summarized in Table 1.

Cytogenetic analysis was performed with the conventional banding technique after 24–48 h incubation and metaphases were evaluated according to the International System for Human Cytogenetic Nomenclature [10]. A karyotype was

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Table 1
Patient characteristics at onset ($n = 138$).

De novo	110
Secondary	28
FAB subtype	
M0–M1	45
M2	27
M4–M5	66
Age years (median, range)	56 (16–84)
Age > 55 years	71/138 (51%)
WBC ($\times 10^9$/L) (median, range)	10.7 (0.9–265)
WBC $\geq 30 \times 10^9$/L	60/138 (43%)
Cytogenetics	
Favorable	13
Intermediate (normal)	48
Intermediate (other)	24
Unfavorable	21
Not done	32
FLT3-ITD	33/118 (28%)
FLT3-TKD	14/118 (12%)
CD34+	80/138 (58%)
CD56+	30/138 (22%)
Bcl2+	40/138 (29%)
MDR-protein expression	
Total negative	44/138 (32%)
PGP+	40/138 (29%)
MRP+	54/138 (39%)
BCRP+	67/138 (48.5%)
Only BCRP+	27/67
BCRP+/PGP+	7/67
BCRP+/MRP+	14/67
BCRP+/PGP+/MRP	19/67

considered normal if at least 20 metaphases without clonal aberrations were observed. Risk group assignment was made according to the MRC criteria [11]. Patients with normal karyotype, but M3 morphology and/or molecular evidence of PML-RARα rearrangement, were excluded from the study.

Total RNA was analysed for identification of the ITD e D835 mutations of the FLT3 gene, as described by Noguera et al. [12], in 118 patients. Multidrug resistance protein (PGP, MRP1, and BCRP) expression was evaluated by flow cytometry as previously described [6]: a MFI ≥ 6 for PGP and ≥ 3 for MRP1 were used as cut-off to distinguish over-expressing cases. In case of BCRP the Kolgomorov–Smirnov test [13] was applied to calculate the *D*-value (i.e. the maximum difference between the test and its negative control). Cases with *D*-value ≥ 0.3 were considered over-expressing BCRP, on the basis of expression in normal peripheral blood cells and bone marrow cells.

All 138 patients were enrolled in one of the Institutional AML protocols, approved by the Ethics Committee, which include fludarabine as part of induction chemotherapy (FLAI/FLAIE). Patients received a first course with fludarabine (25 mg/sqm/d on days 1–5), cytarabine (2 g/sqm/d on days 1–5) and idarubicin (6 or 10 mg/sqm/d on days 1, 3 and 5) with or without etoposide (100 mg/sqm/d on days 1–5). All patients received at least one consolidation course including high-dose cytarabine and idarubicin. Patients considered at high risk of relapse for disease characteristics at diagnosis or poor response to induction therapy were considered candidates for allogeneic stem cell transplantation from sibling or unrelated donors.

Statistical analysis was performed by NCSS software (NCSS, Kaysville, UT, USA).

The association between biological variables was evaluated by regression analysis. The probability of each variable to affect complete remission after induction therapy was calculated by univariate and multivariate logistic regression analysis. Comparison of frequencies between groups was evaluated by two-sided chi-square test. Survival curves were obtained by Kaplan Meier method [14] and different groups were compared by log-rank test. The correlation between different variables affecting survival was evaluated by multivariate Cox regression [15]. Disease free survival (DFS) was defined as the time from complete remission to relapse. Overall survival (OS) was defined as the interval from diagnosis to death, independently of the cause. Patients who underwent allogeneic stem cell transplantation were censored at the time of transplant. *p* values <0.05 were considered statistically significant.

3. Results and discussion

The expression of MDR-related proteins at diagnosis is summarized in Table 1. Forty-four cases (32%) did not over-express any MDR-related protein. PGP over-expression was detected in 40/138 cases (29%), MRP1 in 54/138 (39%) and BCRP in 67/138 patients (48.5%). Among the 67 BCRP-positive cases, 33 showed a BCRP/MRP1 co-expression and 26 expressed both BCRP and PGP; all the three MDR proteins were increased in 19 patients (14%).

Table 2
Factors affecting overall survival (Cox regression analysis).

	RR	95%C.L.	*p*
Age >55 years	3.0	1.63–5.65	0.0004
CD34+	2.7	1.45–5.12	0.0018
BCRP+	2.2	1.26–3.85	0.005

No association was found between high BCRP expression and other clinical or biological characteristic at onset (age, WBC count, cytogenetics, CD34 and CD56 positivity, Bcl-2 over-expression or FLT3 status). However a significantly higher proportion of patients with immature morphology (FAB M0–M1) expressed high levels of BCRP (28/45, 62%) compared to patients with M2 morphology (7/27, 26%; $p = 0.006$) or with monocytic maturation (M4–M5) (32/66, 47%; $p = 0.04$). Moreover, a strong correlation was found between BCRP and PGP ($t = 6.29$, $p = 0.006$) or MRP over-expression ($t = 4.76$, $p = 0.0001$).

Ninety out of 138 patients (65%) obtained a CR after induction chemotherapy. Five (4%) died during induction (DDI) and 43/138 (31%) were primary resistant. The probability to achieve CR was negatively associated to advanced age (>55 years), CD34 positivity, unfavorable cytogenetic and FLT3 internal tandem duplication. No other factors, and in particular over-expression of MDR-related proteins (PGP, MRP1 or BRCP) affected CR rate. All the factors maintained their statistical significance also in the multivariate analysis.

Relapse occurred in 30 of the 90 patients who achieved a CR (33%), at a median time of 12 months (range: 3–36). None of the factors affecting remission achievement had an influence on relapse. However, relapse rate was higher in patients over-expressing BCRP (20 relapses in 45 cases), compared to BCRP-negative patients (10 relapses in 45 cases) (44.5% vs 28.5%, $\chi^2 = 4.05$, $p = 0.04$). Moreover, relapse occurred earlier in the BCRP+ group than in the BCRP- patients (median time to relapse 6 months vs 15 months, log-rank test $\chi^2 = 6.02$, $p = 0.01$) (Fig. 1a). Considering the impact of BCRP and PGP co-expression on relapse, DFS was significantly associated with number and type of over-expressed proteins ($\chi^2 = 8.0$, $p = 0.04$). Both in the BCRP-positive and negative cohort the expression of high PGP levels was associated with a higher probability of relapse, even if in the BCRP+ group the difference between PGP+ and PGP− cases did not reach statistical significance ($\chi^2 = 6.03$, $p = 0.09$). FLT3-ITD mutation alone did not affect DFS, however we observed an additive influence of BCRP over-expression and FLT3-ITD on remission duration: the subgroup of patients BCRP-positive and ITD-mutated have a shorter DFS compared to those with only BCRP over-expression or with only FLT3-ITD or double negative patients ($\chi^2 = 7.26$, $p = 0.02$).

Overall survival was affected by age, unfavorable cytogenetics and CD34 blast expression. Among MDR related proteins, BCRP confirmed its negative role. The cumulative survival curve according to low or high BCRP expression is shown in Fig. 1b ($\chi^2 = 5.95$, $p = 0.01$). Once more, a significant impact was observed in case of concomitant PGP and BCRP over-expression ($\chi^2 = 10.2$, $p = 0.01$). As shown in Table 2, in a multivariate Cox regression model, the risk of death was more than two times higher in case of BCRP over-expression, similar to that of advanced age and CD34 positivity.

Although it is now well known that drugs efflux is only one of the mechanisms by which PGP affects cell function, there is strong evidence that PGP confers resistance to many anti-neoplastic drugs by preventing a sufficient intracellular accumulation. The disappointing results in AML treatment may be due also to the fact