Expression of the Multidrug Resistance-Associated P-Glycoprotein (P-170) in 59 Cases of De Novo Acute Lymphoblastic Leukemia: Prognostic Implications

By Jean E. Goasguen, Jean-Marc Dossot, Olivier Fardel, Franseza Le Mee, Edouard Le Gall, Robert Leblay, Pierre Y. LePrise, Jacques Chaparón, and Renee Fauchet

Immunocytochemical detection of the multidrug resistance (MDR)-associated membrane protein (P-170) was performed at time of diagnosis in a series of 36 children and 23 adults with acute lymphoblastic leukemia (ALL) using two monoclonal antibodies JSB1 and C219. Immunophenotypes were obtained in all cases and karyotypes were analyzed in 37 cases. Detection with JSB1 or with C219 led to similar results in terms of positive cells and cases, but the intensity of staining was higher with JSB1. In the populations studied, the rate of first complete remission differed between MDR-positive and MDR-negative in adult patients only (56% v 93%, respectively, \(P = .05\)). Of the 18 MDR-positive patients who had presented a first complete remission, 13 (81%) relapsed, compared with 13 of 35 (37%) MDR-negative (\(P = .008\)) patients. A higher rate of relapse among MDR-positive compared with MDR-negative patients was observed in adults and in children taken separately (adults 100% v 46%; children 73% v 32%, respectively). The survival rates (Kaplan-Meier method) were significantly higher in MDR-negative compared with MDR-positive populations as a whole (\(P = .002\)) and among children (\(P = .05\)) and adults (\(P = .03\)) taken separately. Event-free survival curves followed this trend. The percentage of second complete remission was very low in the MDR-positive group (15%) compared with 38% for the MDR-negative group. These results were shown by multivariate analysis to be independent of age, immunophenotypes, and karyotypes and clearly show the importance of MDR phenotype detection in ALL.

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**Table 1. Comparison Between Immunologic ALL Subtypes and the MDR Phenotype**

<table>
<thead>
<tr>
<th></th>
<th>Pre B</th>
<th></th>
<th>B</th>
<th></th>
<th>T</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>DR</td>
<td>CD19</td>
<td>CD10</td>
<td>Cu</td>
<td>IgG</td>
<td>CD71</td>
</tr>
<tr>
<td>MDR+</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>MDR-</td>
<td>1</td>
<td>2</td>
<td>25</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

Comparison between "B + pre B" v "T" is not significant, but comparison between B-ALL v pre-B-ALL is statistically significant (P = .01).

Abbreviation: Biph, ALL with myeloid markers.

(generous gift from J.P. Marie, Hotel Dieu, Paris, France). Patients with more than 1% positive cells were considered as positive.

**Statistical analysis.** All statistical calculations were performed according to EPI-INFO program (Epidemiology Program Office, Centers for Disease Control, Atlanta, GA) to study the relationships between P-170 protein expression and complete remission rates, relapse, and second complete remission. The chi-square test (or Fisher's exact test when considering fewer than five patients) was used to compare qualitative parameters (number of complete remissions, number of relapses, and number of second complete remissions). Remission and total survival duration were studied by survival curves according to the Kaplan-Meier method. Patient subgroups were compared by the logrank test. Curves and their comparison were achieved by PCSM software (from Delta Soft France). Multivariate analysis was performed using the Cox regression model (BMDP-2L program) to analyze the simultaneous effect of the different variables. The variables for which univariate analysis had shown a significant association were studied.

**RESULTS**

**Diagnosis.** The population studied included 36 children (median age = 5.2 years, sex ratio F:M = 0.64) and 23 adults (median age = 40.8 years, sex ratio F:M = 1.30). All cases were negative for the myeloperoxidase reaction, and diagnoses were confirmed by immunophenotyping which demonstrated 7 cases of B-ALL, 41 cases of pre-B-ALL subtype, 10 cases of T lineage, and only 1 patient with biphenotypic (lymphoid and myeloid) expression.

**P-170 cellular expression.** We classified those cases with 1% or more positive cells as being positive cases, as modified slightly from the series of Musto et al.\(^{19}\) In the population as a whole, 21 of 56 (38%) patients expressed the MDR protein, with two types of cellular expression observed. Six cases with less than 11% positive cells (1%, 3%, 5%, 7%, 10%, and 10%, respectively) showed a strong reaction of the entire cell with many thick granules, making these cells very easy to count. Four of these 6 cases (66%) presented complete remission, followed in 3 by relapse. The remaining 15 cases with more than 11% cells (36% to 100% in our series) demonstrated many thin red granules with a diffuse light coloration of the cytoplasm observed in some cases. Twelve of these 15 cases (80%) presented complete remission, followed in 10 by relapse. The differences in remission and relapse rates between these two populations are not significant.

Consequently, 17 of the 59 cases were considered as positive for JSB1 and C219, 3 children were positive for JSB1 only, and 1 adult was positive for JSB1 only (C219 expression not performed).

The same percentage of JSB1 or C219 positive cells in association with the same type of positive reaction was observed in 15 cases. Two cases had a higher percentage of JSB1 positive cells compared with C219, one of which presented weaker JSB1 reaction intensity as compared with that for C219.

Hence, the results of P-170 expression shown by these two antibodies (JSB1 and C219) were very similar. The clinical correlations with respect to JSB1 and C219 expression were shown to be very similar as well. For these reasons, in the following presentation the results will be discussed in terms of MDR-positive or -negative, based on JSB1 results, as has been done in previous publications.\(^{2-18}\)

**Immunology.** We found no correlation between P-170 expression and the immunologic subtypes of the 59 ALL (Table 1). However, of the 7 B-ALL (with membrane Ig) were MDR-positive, and the distribution of MDR-positive cases between "B" and pre-B-ALL was statistically significant (P = .01).

**Cytogenetics.** Karyotypes were obtained in 37 cases, 9 of which were MDR-positive and 28 were MDR-negative. Of the MDR-positive, 5 had a normal karyotype and 4 were abnormal, 2 had a translocation [t(9;22) or t(4;18)] associated with a normal number of chromosomes, and 2 had a complex karyotype with hyperdiploidy. Of the MDR negative, 24 had normal karyotypes, and 4 were abnormal with one translocation (4;15) and 3 cases of hyperdiploidy. Even though the difference in the number of karyotype abnormalities between the MDR-positive and -negative population is not statistically significant (P = .07), it appears that the MDR-positive phenotype is more often associated with an abnormal karyotype (44% vs 14%).

**Complete remission, relapse, and second complete remission.** Results are shown in Table 2. In the MDR-positive population complete remission was obtained in 11 of 12 children (92%) and in 5 of 9 adults (56%). In the MDR-negative population the complete remission rate was equal to 92% (22 of 24) and 93% (13 of 14) for children and adults, respectively. The difference concerning adults is statistically significant (P = .05). Relapse was observed in 8 of the 11 MDR-positive children (73%) and in 7 of the 22 (32%) MDR-negative children, the difference being statistically significant (P = .02). Each of the 5 MDR positive adults relapsed,

**Table 2. Results of First Complete Remission (RC1), Relapse, and Second Complete Remission (RC2) According to the MDR Phenotype**

<table>
<thead>
<tr>
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<th>RC1</th>
<th>P</th>
<th>Relapse</th>
<th>RC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children MDR+</td>
<td>11/12 (92)</td>
<td>NS</td>
<td>8/11 (73)</td>
<td>.02</td>
</tr>
<tr>
<td>Adult</td>
<td>5/9</td>
<td>.05</td>
<td>5/5 (100)</td>
<td>.05</td>
</tr>
<tr>
<td>All</td>
<td>16/21 (76)</td>
<td>NS</td>
<td>13/16 (81)</td>
<td>.008</td>
</tr>
<tr>
<td>Children MDR-</td>
<td>22/24 (92)</td>
<td>7/22 (32)</td>
<td>4/7 (57)</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>13/14 (93)</td>
<td>6/13 (46)</td>
<td>1/6 (17)</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>35/38 (92)</td>
<td>13/35 (37)</td>
<td>5/13 (38)</td>
<td></td>
</tr>
</tbody>
</table>

The rate of RC1 in adults is significantly different with the MDR phenotype (56% vs 93%). The rate of relapse is always significantly different (73%, 100%, 81% vs 32%, 48%, and 37%, respectively, for children, adults, and the whole population). Statistical analysis is not available for the RC2 study because of the small number of patients.

Abbreviation: NS, statistical comparison between appropriate respective populations (MDR+ versus MDR−) is not significant.
whereas only 6 of 13 MDR-negative adults relapsed ($P = .05$). In the population as a whole, 13 of 16 (81%) MDR-positive cases relapsed compared with 13 of the 35 (37%) MDR-negative cases ($P = .008$).

A second complete remission was obtained in 2 of 13 (15%) MDR-positive patients and in 5 of 13 (38%) MDR-negative cases.

**Survival and event-free survival (EFS).** Survival and EFS were studied by comparing survival curves (Kaplan-Meier method) by the logrank test. Results are shown in Fig 1. Survival and EFS curves for the whole population and for children in MDR-negative cases are greater than 50% at follow-up of 72 months. In all cases, the survival rates of MDR-positive patients are significantly higher than those of MDR-negative patients.

**Independence of the MDR phenotype.** A stratified univariate analysis of age (<5 years, 5 to 30 years, and >30 years, according to the age distribution) demonstrated that the distribution of MDR-positive or -negative cases was not related to age ($P = .058$). Multivariate analysis using the Cox regression model including age, survival, remission duration, outcome, and leukocyte count showed that the MDR phenotype was an independent factor.

**Comparison between MDR at diagnosis and at relapse.** Only one patient (a 7-year-old child) was tested at diagnosis and at relapse. He was MDR-negative at diagnosis, relapsed after 3.5 years of remission with an MDR-positive phenotype, had a second complete remission (2.5 years), and then relapsed once again without response to treatment. Another patient was tested only at relapse (not included in this series). He was MDR-positive, did not respond to the treatment, and died shortly after.

![Fig 1](image-url)

**DISCUSSION**

The high expression of the MDR1 gene (MDR1-mRNA) has been reported in several human cancers, and more specifically in sarcoma, myeloma, and leukemia. Studies have demonstrated a high expression of the P-170-associated membrane protein in leukemic cells of acute leukemia and in myelodysplastic syndromes, always in limited series and without clinical correlations. We report here a study of 59 ALL patients (36 children and 23 adults) who underwent at diagnosis P-170 detection by an immunocytochemical assay using two different MoAbs (C219 and JSB1). The goal of our study was to determine the effects of MDR on the prognosis of ALL.

Several studies based on the evaluation of MDR1-mRNA have shown a correlation between MDR and resistance to treatment. Rothenberg et al. reported a high level of MDR1-mRNA in 4 of 28 ALL, 1 patient having been tested at diagnosis and the other 3 at relapse. Rothenberg et al.'s study concluded that overexpression without amplification of MDR1-P-170 may be one mechanism of clinical drug resistance in ALL. A second study by Goldstein et al. of more than 400 human cancers reported the presence of MDR1-mRNA expression, particularly in 16 adults and 29 children with ALL. Of the 20 children and 15 adults tested at time of diagnosis, elevated MDR1-mRNA levels were found in 15 and 3 of the cases, respectively. In the same study the relationship with chemoresistance was established and as a result the investigator recommended that MDR1 gene expression be sought when selecting patients for protocols. Marie et al. found in MDR1-mRNA studies on 5 ALL, 23 acute myeloid leukemia (AML), and 13 secondary leukemias that the over-
expression of the gene contributes to chemoresistance. When comparing MDR1 expression in patients with acute leukemia at presentation or at relapse, Herweijer et al. showed a higher expression for treated patients and concluded that the resistance phenomenon was acquired. In their series, 5 of 9 “T” ALL had low levels of MDR1-mRNA, and their two “B” ALL were positive as well. They concluded that MDR1 expression can be found in all subtypes of leukemia and has implications for clinical therapy. Finally, in a series of 63 adult AMLs (median age = 56 years), Pirker et al. showed that high levels of MDR1-RNA lowered complete remission (89% vs 53%, P = .008), disease-free survival (P = .029), and overall survival (P = .009) rates.

These studies on MDR1-mRNA in ALL and in AML all come to the conclusion that the overexpression of the gene is probably and partially implicated in chemoresistance phenomena observed in leukemia therapy. This phenomenon can be expressed at diagnosis and can also be acquired after treatment.

To elucidate the accurate role of the P-170 efflux pump, many investigators have focused their studies on the P-170 cytoplasmic-membrane antigen. The first was Tsuruo et al., who used the MRK16 MoAb on six chronic myeloid leukemia (CML) blast crisis (one ALL). Three were P-170-positive, all with high expression of MDR1-mRNA. In 1990, Kuwazuru et al published three studies concerning MDR1-P-170 detected by C219 and immunoblot technich on 11 ALL,13 11 CML blast crisis (Philadelphia positive),25 and 25 adult “T” leukemia,14 which demonstrated the relationship between the presence of the P-170 and drug resistance (non-response to treatment or relapse). In Kuwazuru et al’s study on ALL, four patients were tested at diagnosis and at relapse. One case initially positive was still positive at relapse and did not respond to the treatment. One case initially negative at diagnosis and positive at relapse did not respond to treatment, and of 2 cases that were negative at diagnosis and then relapsed, 1 did not respond to treatment and the second had only a partial response.

These results clearly showed the clinical significance of the MDR phenomenon. Only one study28 using MRK16 on 19 leukemic cases, of which only 5 were ALL, did not find a clinical correlation.

Our study reports here the largest series ever of ALL cases examined on the basis of the P-170-associated membrane protein with a follow-up of 1 to 72 months. As shown in Table 2, the most important result is the significantly higher relapse rate (81%) in the two MDR positive subpopulations as a whole (children and adults) compared with the MDR-negative population (37%, P = .008). Of the 59 patients at diagnosis, 51 presented with first complete remission, 26 of whom relapsed. Thirteen of these 26 patients were MDR-positive at diagnosis. This result leads to the conclusion that 50% of relapses can be predicted by MDR phenotype, and studies should be undertaken to focus on the factors that may lead to relapse in the other 50% of patients. These results can be compared with those of Kuwazuru et al., who found 6 of 6 ATL-MDR-positive at relapse.

The second point of our study is the likelihood of a lower rate of second complete remission in MDR-positive patients (15%) compared with MDR-negative cases (38%). These rates are not significantly different (P = .07) in this study, most likely due to the small number of patients present after relapse, especially in MDR-positive patients. Therefore, the clinical significance of this observation can only be suggested. It would be interesting to confirm by molecular biology the hypothesis of Herweijer et al.10 and Goldstein et al.11 of acquired P-170 cytoplasmic membrane expression after chemotherapy.

Finally, the least demonstrative results in our study concern the differences in first complete remission rates. In the pediatric population, MDR-positive and MDR-negative patients have exactly the same rate of CR (92%), whereas only 56% of MDR-positive adults presented with CR compared with 93% of MDR-negative cases (P = .05). Age cannot explain this difference as demonstrated by the multivariate analysis, and therefore the hypothesis that the adult ALL population is heterogeneous must be discussed. Immunophenotype cannot be implicated either, because of the 7 cases of “B” ALL (with IgS), all were adults, 6 of whom were MDR-positive in which only 2 did not present CR. Cytogenetic analysis does not explain the observed heterogeneity because the karyotype abnormalities shown in our study do not correlate with the MDR phenomenon. Three of the 8 MDR-positive cases had abnormal karyotypes compared with 4 of the 21 MDR-negative cases, this difference being nonsignificant and very similar to those found in the pediatric group.

One MDR-positive patient was 47,XY,t(14;18), + 7. This observation can be compared with that of Redner et al., who demonstrated a high expression of the P170 in a near-haploid case. Because the MDR1 gene is located on chromosome 7, due to the abnormalities shown in our study do not correlate with the MDR phenomenon, three of the 8 MDR-positive cases had abnormal karyotypes compared with 4 of the 21 MDR-negative cases, this difference being nonsignificant and very similar to those found in the pediatric group.

One patient with de novo ALL had a t(9;22), was MDR-negative, presented with complete remission, had a BM transplantation, and is surviving 31 months later without relapse.

The low complete remission rate observed in MDR-positive adults needs to be confirmed in a larger series to clarify the differences with the pediatric series. The total survival and EFS curves differ greatly between MDR-positive compared with MDR-negative patients. The differences found for the children are amplified in comparison with those of the adults who died more quickly. The difference of survival by age is highly significant (P = .0001) and this fact is already well known in ALL. Furthermore, multivariate analysis shows that the MDR phenotype is independent of age. To date, our study is the first on ALL comparing survival or remission duration according to MDR phenotype. We can compare our results with those obtained by Campos et al.11 on 150 AML cases, who found a significant (P = 10^-5) difference between MDR-positive (4 months) and MDR-negative patients’ median survival (15 months) as well as with those of Pirker et al.23

In conclusion, several studies have already shown the implication in leukemias of the MDR gene in the failure of drug protocols. These reports were very limited by the low number of patients, and in part by cumbersome molecular biology technology. The use of MoAbs directed against the P-170 membrane protein is easier and can be performed in routine.
Our results clearly show that the MDR-positive phenotype is associated with a higher relapse rate, a shorter remission duration, and a shorter median survival compared with the MDR-negative phenotype. This observation is independent of age, leukocyte count, immunophenotype (except for ALL with membrane Ig), and karyotype.

REFERENCES


Expression of the multidrug resistance-associated P-glycoprotein (P-170) in 59 cases of de novo acute lymphoblastic leukemia: prognostic implications [see comments]

JE Goasguen, JM Dossot, O Fardel, F Le Mee, E Le Gall, R Leblay, PY LePrise, J Chaperon and R Fauchet