Clinical Significance of Multidrug Resistance P-Glycoprotein Expression on Acute Nonlymphoblastic Leukemia Cells at Diagnosis

By Lydia Campos, Denis Guyotat, Eric Archimbaud, Pascale Calmard-Oriol, Takashi Tsuruo, Jacques Troncy, Danielle Treille, and Denis Fiere

To evaluate the clinical value of the expression of multidrug resistance P-glycoprotein (P-170) on the surface of acute nonlymphoblastic leukemia (ANLL) cells, we analyzed specimens from 150 newly diagnosed patients for staining with MRK16, a monoclonal antibody (MoAb) that binds to an external epitope of P-170. Other surface markers (CD13, CD14, CD15, and CD34) were studied by the same technique. A marker was considered positive when 20% or more cells were stained. Of 150 samples, 71 were P-170-positive. These cases did not differ from P-170-negative cases with regard to age, sex, initial white blood cell (WBC) counts, or French-American-British (FAB) type (except for M3 ANLL, which were more frequently negative). However, leukemias arising from previous myelodysplastic syndrome (MDS) and therapy-induced leukemias were more frequently P-170-positive. CD34 and P-170 expression were significantly associated. All patients were treated by intensive chemotherapy. Complete remission (CR) rates were significantly lower in P-170-positive (23/71, 32%) than in P-170-negative cases (64/79, 81%) (P < 10^-4). CD34 positivity was also associated with a low remission rate (P < 10^-4). Survival was shorter for P-170-and CD34-positive patients (P < 10^-4). The prognostic value of both markers was confirmed in multivariate analysis. CR duration was also shorter for P-170-positive cases, but the difference is less significant (P = .05). It is concluded that P-170 analysis may be an important tool for predicting the outcome of intensive chemotherapy in ANLL patients.

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TABLE 1. Chemotherapy Protocols Used

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Duration</th>
<th>Course 1</th>
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</thead>
<tbody>
<tr>
<td>LYLAM protocol (75 patients, age ≤ 60)</td>
<td></td>
<td>Daunorubicin, 70 mg/m², d 1 to 3</td>
</tr>
<tr>
<td>Course 1</td>
<td></td>
<td>Cytosine arabinoside, 200 mg/m², d 1 to 7</td>
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<td></td>
<td></td>
<td>6-Thioguanine, 200 mg/m², d 1 to 7</td>
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<td></td>
<td></td>
<td>Maintenance</td>
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<tr>
<td></td>
<td></td>
<td>Low-dose cytosine arabinoside for 12 mo</td>
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<tr>
<td>EORTC AML9 (44 patients, age &gt; 60)</td>
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<td>Daunorubicin, 30 mg/m², d 1 to 3</td>
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<tr>
<td>Course 1</td>
<td></td>
<td>Mitoxantrone, 8 mg/m², d 1 to 3</td>
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<tr>
<td></td>
<td></td>
<td>Cytosine arabinoside, 100 mg/m², d 1 to 7</td>
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<tr>
<td></td>
<td></td>
<td>Maintenance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low-dose cytosine arabinoside or no maintenance</td>
</tr>
</tbody>
</table>

CRIS-6 (a gift from Dr Vilella, Barcelona, Spain) directed to CD14 antigen; SMy15a (Biosys, Compiègne, France) to CD15 antigen; My10 (Becton Dickinson) to CD34. Human AB serum was added to the cell suspension to avoid nonspecific fixation of the MoAb on Fc receptors. Cells were incubated with MoAbs at 4°C for 30 minutes, washed with PBS, incubated with F(ab'') fragments, and analyzed by indirect immunofluorescence. Controls were performed with a nonreactive antibody (MsIgM or MsIgG kits, Coulter Immunology). The staining was considered positive when 20% more cells than the control were stained.

**Statistical analysis.** The relationships of the expression of P-170 to quantitative parameters (age, blood counts, percent of marrow blasts) were studied by analysis of variance. The relationships to quantitative parameters (sex, FAB type, extramedullary disease remission) were analyzed by chi-square test (or Fisher’s exact test when either group included fewer than 20 cases).

Survival curves were plotted according to the method of Kaplan-Meier. Survival or remission durations of different groups were compared by the log-rank test. The respective influence of different parameters on survival or CR duration was calculated according to the Cox proportional hazards regression method. For this last analysis, the expression of P-170 and other surface markers was used as a continuous variable, and a logarithmic transformation of white blood cell (WBC) count was performed.

All calculations were performed using the BMDP statistical program (BMDP Statistical Software, Los Angeles, CA).

**RESULTS**

**P-170 expression.** The expression of P-170 was heterogeneous in terms of number of cells stained or fluorescence intensity. Figure 1 shows the number of samples according to the percentage of positive stained cells. With a cutoff point of 20%, 71 of 150 samples studied (47%) were considered positive for P-170 expression.

**Correlations with clinical and biological characteristics.** There was no correlation between P-170 expression and initial characteristics such as sex, age, presence of extramedullar disease, blood counts, or marrow blast percentage.

No relationship was noted for FAB subtype, with the exception of M3 subtype, where only two of 12 cases were P-170-positive (P < .05).

One hundred twenty-two ANLL cases were considered as de novo, while 12 cases were secondary to preceding MDS and 16 to chemotherapy for previous malignancy. P-170 was more frequently expressed in ANLL secondary to MDS (9/16) and previous chemotherapy (10/16) than in de novo ANLL (52/122) (P < .05).

The relationships of P-170 to other surface markers were studied. CD13, CD14, CD15, and CD34 were positive in 121 (81%), 60 (40%), 114 (76%), and 83 (55%) samples, respectively. There was a positive correlation between P-170 and CD34, as 96 of 150 samples had similar patterns of staining for both markers (50 positive and 46 negative) (P < .001).

The same overall correlation was found using the percentages of stained cells as continuous variables (r = .31, P < 10^-4). There was no significant correlation with other surface markers.

**Prognostic value of P-170 expression.** CR was obtained in 87 of 150 patients (58%). The remission rate was highly predicted by P-170 expression, as 23 of 71 (32%) P-170-positive patients obtained remission versus 64 of 79 (81%) P-170-negative patients (P < 10^-3). The remission rate was also influenced by age (CR rate 54/75 for patients aged 60 or less v 33/75 for patients over 60, P < 10^-3), WBC count (CR rate 54/78 for WBC ≤ 30 x 10^9/L v 33/72 for WBC > 30 x 10^9/L, P < .01) and preceding MDS or therapy-induced ANLL (CR rate 77/122 for de novo ANLL v 10/28 for secondary ANLL, P < .01). The influence of P-170 remained significant within each of these prognostic categories, in younger (P < .005) and older (P < 10^-4) patients, in cases with (P < 10^-4) or without hyperleukocytosis (P < 10^-3), and in de novo ANLL (P < 10^-3).
CD34 expression was also predictive of induction treatment outcome, as 28 of 83 (34%) CD34+ patients went into remission, versus 59 of 67 (88%) CD34- patients \( (P < 10^{-4}) \). By combining both markers, it was possible to define a subgroup with a very poor prognosis (both markers positive, CR rate 9/50) and a subgroup with very good prognosis (both markers negative, CR rate 45/46).

Factors influencing survival were first studied by univariate analysis. Survival was significantly shorter for CD34+ \( (P < 10^{-5}) \) and P-170-positive patients \( (P < 10^{-5}) \) (Fig 2). Age 60 or less was also significant \( (P = .01) \). In multivariate analysis, the significant variables were P-170 \( (P < .005) \) and CD34 expression \( (P < .001) \), and age \( (P < .01) \). The presence of preceding myelodysplastic syndrome or secondary leukemia did not significantly influence survival, nor did initial counts, FAB classification, or other surface markers.

The remission duration was studied in 87 patients by univariate analysis, there was no significant factor, but patients with de novo disease, and with P-170-negative disease showed a trend toward longer remission \( (.05 < P < .1) \). In multivariate analysis, P-170 expression significantly influenced CR duration \( (P = .05) \).

**DISCUSSION**

The expression of MDR-1 products has already been reported in a large number of ANLL patients, mainly in refractory or relapsed disease, but also at diagnosis. The percentage of untreated cases expressing MDR1 obviously depends on the method used for detection. In our study using MRK16 MoAb and the staining technique described by Hamada and Tsuoru, we observed 47% positive cases. This is consistent with the results of Sato et al, who reported a high expression of MDR-1 RNA transcripts in 36 untreated patients. This percentage is much higher than that reported by Kennitz et al on a larger series of patients using a polyclonal antibody and an immunocytochemical technique. Like Sato et al, we did not observe a correlation with initial characteristics such as age, hematological parameters, or FAB classification (the exception of FAB M3), and we confirmed the higher expression in therapy-related leukemias. This last finding might be explained by previous exposure to chemotherapy, but we also observed a high expression in ANLL secondary to MDS, which confirms the observation of Holmes et al. It is possible that P-170 is expressed more in leukemias arising from poorly differentiated cells. The partial correlation with CD34 is consistent with this hypothesis, and was recently reported by List et al in a series of 45 cases with MDS and therapy-induced ANLL.

High levels of MDR-1/P-170 in malignant cells have been associated with clinical resistance to chemotherapy in a variety of malignancies, including ovarian cancer, neuroblastoma, myeloma, and acute leukemia. However, few prospective studies have been conducted in patients with newly diagnosed ANLL. In the series of Sato et al, a higher CR rate and a longer remission duration were observed in patients with low levels of MDR-1 transcripts. We also observed a significant difference between P-170-positive and -negative patients with regard to CR achievement and survival. The difference was less significant for CR duration, but this may be explained by the fact that there were too few patients in the P-170-positive group in remission, and by the heterogeneity of postinduction treatments. Our results are in contradiction with those of Ball et al, although we used the same MoAb to detect P-170. Detailed analysis of this last study is needed to understand this discrepancy. Our study also confirmed the prognosis value of CD34 that we and others have already reported.

Although our data indicate that P-170 expression is highly correlated with therapy outcome, MDR is certainly not the only factor of drug resistance in ANLL. However, in vitro data show that drug resistance of ANLL cells to daunorubicin may be reversed by P-170 blockers such as verapamil. Our study contributes to the identification of patients with poor prognosis, but also suggests that therapeutic trials using P-170 blockers in addition to chemotherapy might be of interest, at least for patients with MDR-1-positive blast cells.

**REFERENCES**

6. Carulli G, Petrini M, Marini A, Ambrogi F: P-glycoprotein in...


Clinical significance of multidrug resistance P-glycoprotein expression on acute nonlymphoblastic leukemia cells at diagnosis [see comments]

L Campos, D Guyotat, E Archimbaud, P Calmard-Oriol, T Tsuruo, J Troncy, D Treille and D Fiere