# P-Glycoprotein Expression in Acute Myeloid Leukaemia Cells at Diagnosis: Its relationship to Daunorubicin or Idarubicin Induction Therapy and Survival

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## Malignancy

# P-Glycoprotein Expression in Acute Myeloid Leukaemia Cells at Diagnosis: Its relationship to Daunorubicin or Idarubicin Induction Therapy and Survival

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We investigated the expression of P-glycoprotein (P-gp) in 50 adults with *de novo* diagnosed acute myeloid leukaemia (AML) and the relationship between presence of P-gp in leukaemic cells and efficacy, as remission induction and survival rate, of two different anthracyclines, daunorubicin (DNR) and idarubicin (IDR).

We found that 30 out of 50 patients (60%) were negative (Group 1) and 20 (40%) were positive (Group 2) for P-gp expression evaluated by mean of MRK16 MoAb using a cut-off of 10% positive cells. Thirty-five out of 50 patients (70%) obtained complete remission (CR); depending on P-gp expression, the CR rate was 80% for group 1 and 45% for group 2 ($p < 0.005$).

The median duration of overall survival was 20 months for patients in Group 1 as compared with 10 months for patients of Group 2 ($p < 0.005$).

Regarding the anthracycline used, no significant difference in CR was observed in patients of Group 1 (75% of CR with DNR vs. 90% with IDR); Group 2 obtained 40% of CR with DNR vs. 70% with IDR ($p < 0.005$). The median duration of overall survival (OS) with the two regimens was comparable in Group 1, while it was significantly longer in patients of Group 2 treated with IDR compared with DNR regimen ($p < 0.005$).

These results confirm the prognostic value of P-gp expression in AML at first appearance and we suggest that idarubicin could be a valid anthracycline drug in the treatment of AML to be evaluated as potential drug of choice in patients with primary or drug-induced multidrug resistance.

*Keywords*: P-glycoprotein, acute myeloid leukaemia, daunorubicin, idarubicin

## INTRODUCTION

Although the last 20 years have seen dramatic advances in chemotherapy treatment of

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haematological malignancies, certain obstacles remain.

Probably the most important one is the development of resistance to the drugs used for treatment [1]. Primary drug resistance in acute myelogenous leukaemia (AML), for example, is a poorly understood phenomenon although 10 to 20% of adults with disease do not respond to two or more courses of intensive chemotherapy [2–4].

At least two types of mechanisms are involved in the cellular resistance to anticancer drugs. The first one, called multidrug resistance (MDR), is characterised by the overexpression of a membrane high molecular weight glycoprotein known as P-glycoprotein or multidrug transporter and encoded by the MDR1 gene. This protein acts as a pump capable of extruding xenobiotics, especially anticancer drugs of natural origin, out of the cell. Therefore, in multidrug resistant cells there is a cross-resistance between several unrelated drugs such as anthracyclines, Vinca alkaloids or epipodophyllotoxins.

Other mechanisms of resistance to anthracyclines have been described. A second ATP-dependent membrane pump called MRP has been identified in resistant cell lines, and seems to modulate the intracellular distribution of drugs rather than their gross accumulation. Alterations of the common target of anthracyclines and epipodophyllotoxins, DNA-topoisomerase II, also lead to cross-resistance to these drugs. These quantitative and/or qualitative alterations allow the enzyme to escape the formation of the lethal cleavable complex with the drugs, even though little is presently known about the occurrence of this mechanism of resistance in clinics.

Recent studies in patients with de novo and relapsed AML have shown that mdr1 gene expression linked with clinical drug resistance [9,10]. Marie et al. [9] detected the presence of mdr1 gene in 19% of patients with de novo AML and in 50% of patients after relapse. Pirker [10] reported mdr1 gene expression in 70% of patients with de novo AML; the complete remission rate was 53% compared with the 90%

in the group without mdr1 gene expression. Campos [11] reported similar results.

Recently many reports compared treatment with idarubicin (IDR), a new anthracycline derivative, in combination with cytosine arabinoside (ARA-C) to standard daunorubicin (DNR) and ARA-C regimen. Those reports showed that fewer patients on the IDR arm had primary refractory disease after two courses of induction therapy [12,13] than those on standard treatment.

The question has been raised as to whether idarubicin participated in this mechanism of resistance, and several studies have been undertaken. It appeared that idarubicin was only partially cross-resistant to other anthracyclines in multidrug-resistant models. The resistance factors to idarubicin are 10-fold lower than to daunorubicin in P-glycoprotein expressing resistant cells, and similar to both drugs in a non-MDR cell line. This finding has been confirmed by the fact that there was greater accumulation and retention of idarubicin than of daunorubicin, in Vinca alkaloid-resistant leukaemic cells and that verapamil, an MDR modulator, had little effect on these parameters. Many authors examined the effect of the anthracycline drugs in leukaemia cell lines that display the MDR phenotype [14,15]. Results of these studies suggest that IDR may be more effective than DNR in leukaemia cells that display the MDR phenotype [16,17].

The present paper reports our experience to further define the clinical significance of the expression of P-glycoprotein in leukaemia cells at diagnosis and correlate this unfavourable prognostic marker with different anthracycline schedules (DNR and IDR) and clinical outcome.

## PATIENTS AND METHODS

### Patients

From January 1991 to June 1994, 50 patients (23 females, 27 males, aged 18–80 years, median

62 years) with *de novo* AML were enrolled in the study. Remission induction chemotherapy consisted of an anthracycline combined with cytosine arabinoside. Twenty patients received a regimen containing daunorubicin 45 mg/m²/ die in days 1–3, cytosine arabinoside 100 mg/m² in every 12 h days 1–7; 10 patients received DNR + cytosine arabinoside + VP16 100 mg/m² days 1–5. The remaining 20 patients received idarubicin 10 mg/m²/die days 1–3 instead of DNR and cytosine arabinoside 100 mg/m² in every 12 h days 1–7. Patients were casually assigned to receive the regimen containing daunorubicin or that containing idarubicin.

Consolidation therapy consisted of either DNR + cytosine arabinoside ± VP16 for 2 cycles or idarubicin associated to cytosine arabinoside alternated to VP16 for further 2 cycles.

Intensification therapy comprised HD cytosine arabinoside (2 gr/m² in every 12 h) for 5 days in patients <65 years old. In patients >65 years old we considered additional two cycles of chemotherapy likely to consolidation program. No maintenance program was performed in our study. Three patients underwent to alloBMT and four patients to autoBMT after intensification schedule was completed.

### Leukaemic Cells

The 4leukaemic cells were isolated from peripheral blood and/or bone marrow by Ficoll-Hypaque gradient centrifugation, washed and resuspended in Phosphate-Buffered Saline (PBS) or RPMI-1640. Cytospin samples showed that the percentage of blasts was always greater than 80% after separation. Cells were analysed immediately.

### P-170 Expression

P-170 expression was analysed by indirect immunofluorescence, using the MRK16 monoclonal antibody (MoAb) as described by Tsuruo [18]. 1 × 10⁶ cells were fixed in 1% paraformaldehyde/PBS, washed twice and incubated for 30 minutes at 4 °C with 200 μl of MoAb solution (10 μg/ml). Cells were then washed twice in PBS, and fluorescein-conjugated rabbit anti-mouse immunoglobulin IgG serum was added for 30 minutes at 4 °C. Analyses were performed with a FACScan II cytometer (Becton Dickinson).

Including normal mouse serum instead of MRK16 MOAB performed negative controls. Samples were considered positive if 10% cells more than control cells were stained.

### Statistical Analysis

Survival curves were plotted according to the method of Kaplan and Meier [19]. Survival of remission duration of different groups was compared with log-rank test. Analysis was performed using the BDMP Release 7, Dynamic statistical package.

### RESULTS

The expression of P-glycoprotein in leukaemic cells by means of MoAb MRK16 in 50 patients with *de novo* AML was evaluated. The evaluation of the expression of P-gp was heterogeneous in term of number of cells stained or fluorescence intensity. For further analysis, patients were divided into those with 0 to 10% stained cells (Group 1) and those with >10% stained cells (Group 2). Group 1 was composed of 30 patients and Group 2 of 20 patients (Table I). Age and sex of patients, serum lactate dehydrogenase levels and duration of follow-up were different between the two groups. WBC and percentage of blasts were higher in patients of Group 2, but did not reach a statistical significance level (p = 0.09). Chromosomal abnormalities and F.A.B. subgroups did not differ between the two groups, even if data showed a trend to reach a significant difference; we could reasonably hypothesise it could be due to the low number of patients in the various subgroups.

TABLE I    Patients characteristics

|  | Total | Group 1 | Group 2 | *p*-value |
|---|---|---|---|---|
| *P-glycoprotein* |  | 0–10% | >10% |  |
| *Pts (No.)* | 50 | 30 | 20 |  |
| *Age (years)* |  |  |  |  |
| median | 62 | 60 | 66 |  |
| range | 18–80 | 18–80 | 24–78 | ns |
| *Sex (F/M)* | 23/27 | 14/16 | 8/12 | ns |
| *LDH (U/L)* |  |  |  |  |
| median | 420 | 390 | 460 | ns |
| range | 150–3600 | 160–2880 | 145–3900 | ns |
| *WBC ($10^9$/L)* |  |  |  |  |
| median | 12.8 | 9.4 | 24.5 | 0.5 |
| range | 0.8–185 | 0.8–180 | 1.2–186 |  |
| *Blasts (%)* |  |  |  |  |
| median | 60 | 52 | 78 | 0.08 |
| range | 6–98 | 6–96 | 8–99 |  |
| *Cytogenetic abnormalities* |  |  |  |  |
| normal | 32 | 18 | 14 |  |
| others | 6 | 4 | 2 |  |
| complex | 6 | 3 | 3 |  |
| undetectable | 6 | 5 | 1 |  |
| *FAB subgroups* |  |  |  |  |
| M0 | 3 | 1 | 2 |  |
| M1 | 4 | 2 | 2 |  |
| M2 | 22 | 12 | 10 |  |
| M3 | 3 | 3 | 0 |  |
| M4 | 8 | 5 | 3 |  |
| M5 | 6 | 4 | 2 |  |
| M6 | 4 | 3 | 1 |  |

The association of P-gp expression with the outcome of induction chemotherapy was evaluated. Thirty-five out of 50 patients (70%) obtained complete remission (CR) which is in line with the currently achievable CR rate in this disease. Dependent on P-gp expression (Table II), however, the CR rate was 80% in Group 1 and 45% in Group 2 ($p < 0.02$). Within Group 2, the CR rate

was 40% for patients with staining cells between 10–30% and 20% for patients with >30% staining cells (Table II). Early death (within 4 weeks after beginning of treatment) was more frequent in Group 2 than in Group 1 (30% versus 13%, $p < 0.05$). The percentage of resistant disease in our patients was 7% in P-gp <10% versus 15% in P-gp >10% ($p < 0.03$). Age (CR rate higher for patients aged 60 or less) and WBC count (CR rate higher for WBC $< 30 \times 10^9$/L) also influenced the remission rate. The influence of P-gp remained significant within each of these prognostic categories, in younger patients aged less than 60 years (23 out of 50 patients; $p < 0.005$) and in cases without hyperleukocytosis, i.e. WBC count $< 30 \times 10^9$/L, (38 out of 50 patients; $p < 0.001$).

Regarding the different anthracycline regimen used (DNR versus IDR), we observed that in term of CR % no difference has been observed in patients with P-gp <10% staining cells (75% CR with DNR) versus 90% CR with IDR; on the contrary when P-gp staining cells positivity was above 10% DNR treatment reached 40% CR versus 70% CR with IDR ($p < 0.001$ — Table III). Moreover, the percentage of patients with resistant disease (after two cycles of induction therapy) was quite different in the two regimens. Resistance was 10% with DNR versus 10% with IDR with a cell positivity less than 10%; with more than 10%, resistant disease in DNR groups was 30% versus 10% in IDR groups ($p < 0.01$). The duration of survival was calculated in according to Kaplan-Meier [18]. The median duration of observation for the total study population

TABLE II    P-glycoprotein expression of leukaemic cells and outcome of induction chemotherapy

| P-glycoprotein Positive cells (%) | No. of patients (%) | CR (%) (a) | Early death (%) (b) | Resistant disease (%) |
|---|---|---|---|---|
| 0–10 | 30 | 80* | 13** | 7*** |
| >10 | 20 | 45* | 30** | 15*** |
| 10–30 | 15 | 40 |  |  |
| >30 | 5 | 20 |  |  |

a) After 2 cycles of induction regimen.
b) Within 4 weeks after beginning of therapy.
c) *p*-value ($\chi^2$-test): *$p < 0.02$; **$p < 0.05$; ***$p < 0.03$.

TABLE III   CD34 expression, anthracycline containing regimen, P-gp expression and clinical outcome

| P-glycoprotein expression (%) | CD34 expression (%) | | No. of patients | | CR (%) | | Resistant disease (%) | |
|---|---|---|---|---|---|---|---|---|
| | CD34 + | CD34 − | DNR | IDR | DNR | IDR | DNR | IDR |
| 0–10 | 18 | 30 | 20 | 10 | 75 | 90 | 10 | 10 |
| >10 | 10 | 15 | 10 | 10 | 40* | 70* | 30** | 10** |

$p$-value ($\chi^2$-test): *$p < 0.001$; **$p < 0.001$.

was 30 months for Group 1 and 24 months for Group 2. The median duration of overall survival was 16 months for the total study population. Depending on P-gp expression, however, the duration of overall survival was 20 months for patients of Group 1 as compared to 10 months for patients of Group 2 ($p < 0.005$ — Figure 1). Actuarial 5 years of DFS of the P-gp negative patients in CR was $60 \pm 15\%$ and that of the P-gp positive patients in CR was $25 \pm 20\%$

(Figure 2). The DFS of P-gp negative patients was longer than that of P-gp positive patients, but not to a statistically significant extent ($p = 0.08$). Figure 3 shows that in presence of P-gp less 10% median duration of O.S. is not different between IDR and DNR regimen (20 months versus 18 months). On the contrary the median duration of O.S. in patients with P-gp more 10%, treated with IDR regimen, was significantly longer than DNR counterpart
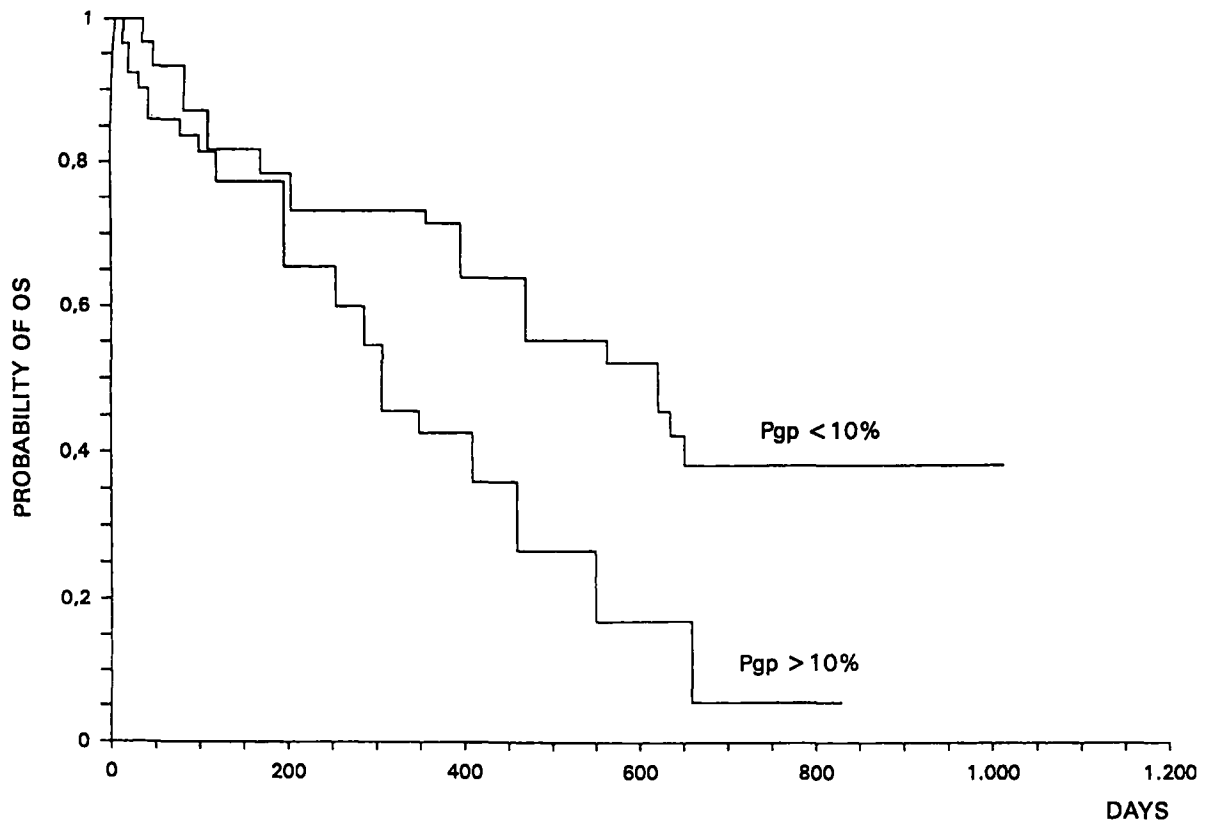


FIGURE 1   For survival analysis according to Kaplan-Mayer, patients were grouped into patients with 1–10% staining cells and patients with >10% staining cells. Based on this division, OS of the P-gp < 10% patients was significantly longer than OS of P-gp >10% patients ($p < 0.005$).
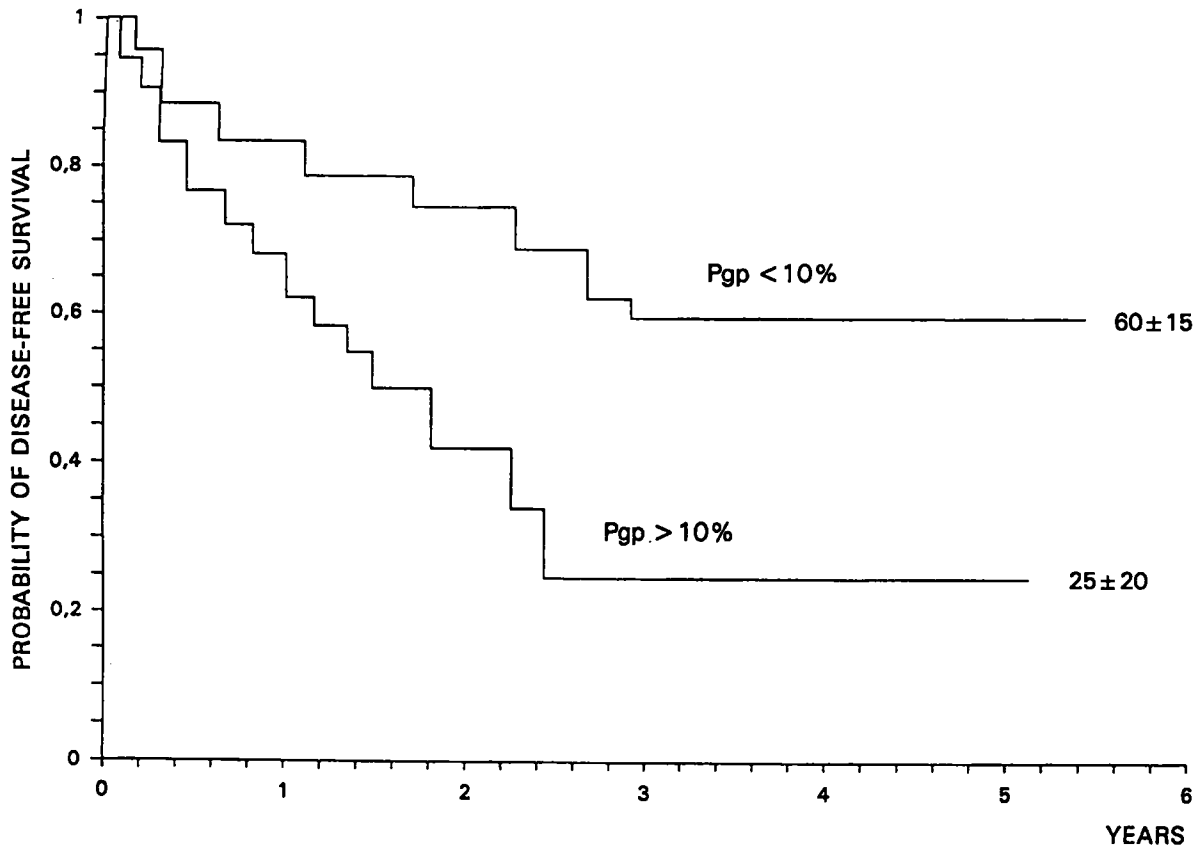
FIGURE 2    Kaplan-Mayer plot of disease-free survival (DFS) P-gp <10% patients and P-gp >10% patients. Actuarial 5-years DFS of P-gp <10% patients was 60 ± 15% and P-gp >10% was 25 ± 20% ($p = 0.08$).

group (18 months versus 8 months, $p < 0.005$). The remission duration in 35 patients who obtained CR, with P-gp less 10% and treated with IDR regimen, showed a trend towards longer remission without reaching significant difference ($p < 0.08$), according to univariate analysis; again, we could reasonably hypothesise it could be due to the low number of patients and to the short follow-up time.

## DISCUSSION

The expression of *mdr1* gene has been well-documented in acute leukaemia both at presentation and at relapse [20–22]; and it represents an independent prognostic factor [23]. In AML,

P-gp expression is more frequent in primary refractory and resistant disease [21], and it has also been described as a marker of early relapse [22], when present during remission period. Several reports have shown that *mdr1* RNA levels and blast membrane P-gp in AML at diagnosis are associated with a lower CR rate and a shorter survival [11,24].

Similarly our findings, based on a flow cytometry method for detecting P-gp (MoAb MRK16), confirm these previous observations. In our experience we determined that 20 (40%) of the newly diagnosed patients with AML were positive for P-gp expression. This percentage is consistent with findings of other researchers who used different methods. Also, we showed both a lower CR rate and a shorter duration of
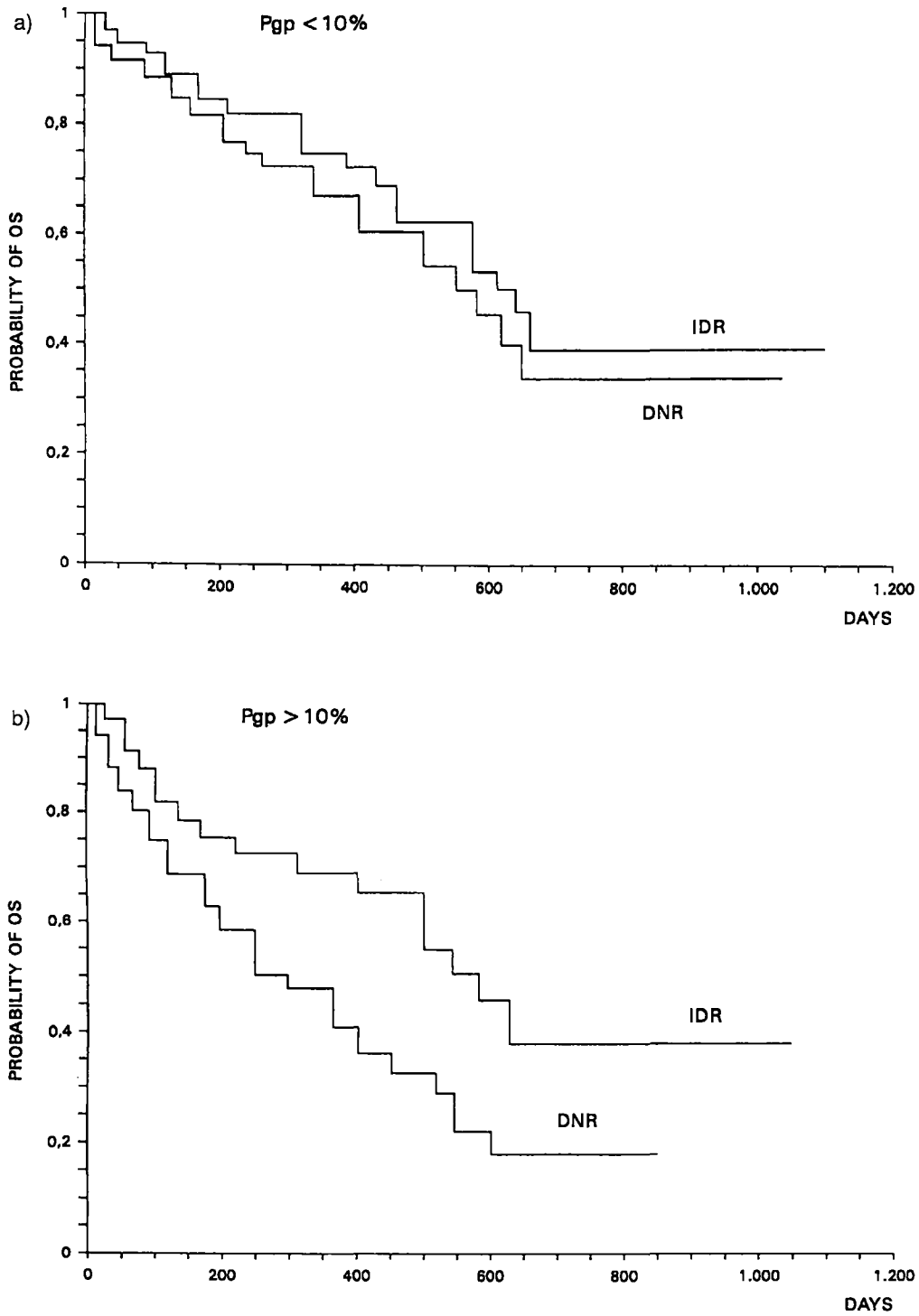
FIGURE 3   Overall survival of IDR and DNR regimen P-gp < 10% patients did not show any difference between IDR and DNR regimen (a). On the contrary OS of P-gp > 10% patients treated with IDR regimen was significantly longer than patients treated with DNR ($p < 0.005$)(b).

overall survival for patients with > 10% P-gp positive cells at diagnosis as compared with patients with 0–10% of positive cells. We were, however, unable to show any association between the expression of CD34 and P-gp; this contrasts with the findings of Campos but it is in agreement with Wood's paper [24]. The discrepancy between our findings and those of others may likely be due to the size of the patient sample studied.

Our study and others indicate that leukaemic cells with primary chemoresistance do exist and multidrug resistance is a clinically important type of drug resistance in AML. However, recently many reports have shown that AML patients treated with idarubicin present a minor incidence of refractory disease compared with patients treated with DNR. Our preliminary study showed AML patients with P-gp > 10% treated with IDR achieved a more significant CR rate as compared to DNR arm ($p < 0.001$). We observed also in IDR arm patients with P-gp > 10% a lower incidence of primary resistant disease as compared to DNR [6,12]. Overall survival in IDR arm associated with P-gp > 10% is better than DNR arm. These preliminary data confirm both *in vitro* data of Berman [16] obtained with leukaemic cell lines and those obtained in randomised clinical trials comparing DNR versus IDR as first line therapy of newly AML patients [12,13].

This data could be explained either by the high lipophilicity of idarubicin, which would allow enhanced passive influx of the drug, or by impaired recognition of idarubicin by P-glicoprotein, which would decrease its active efflux. Regardless of the mechanism, there is a partial overcoming of MDR, which must be emphasised in view of the importance of this mechanism of escaping drug action in haematological malignancies, especially acute myelogenous leukaemia. In this respect, Nussler has shown clinical efficacy of idarubicin in 11 of 15 patients who demonstrated overexpression of P-glycoprotein in their leukaemic cells [25].

Moreover, it has been known for a long time that idarubicin was active in acute leukaemias, non-Hodgkin's lymphoma and multiple myeloma refractory to reference anthracycline treatment. The remission duration analysis in terms of anthracycline arm versus *mdr1* expression did not reach statistical significance in our population probably due to the small size of the patient sample enrolled in the study.

According to several previous reports [7, 26–28], the observed association of *mdr1* gene expression in the poor outcome warrants future evaluation of the clinical usefulness of the determination of gene expression with respect to therapeutic decisions. In this making decision IDR as first line induction therapy, could be a valid anthracycline drug to circumvent the P-gp mediated MDR in AML patients. A large randomised clinical trial would be necessary to validate efficacy and safety of idarubicin in AML settings with worst prognosis.

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