Pergamon

European Journal of Cancer Vol. 32A, No. 6, pp. 1034–1038, 1996

Printed in Great Britain
0959–8049/96 $15.00+0.00

S0959-8049(96)00055-X

MDR1/P-glycoprotein in Haematological Neoplasms

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INTRODUCTION

HAEMATOLOGICAL NEOPLASMS are initially sensitive to chemotherapy, but the relapse rate is usually high, except for Hodgkin's lymphoma and childhood acute lymphoblastic leukaemia (ALL). Therefore, the phenotype of multidrug drug resistance (MDR) is mainly observed after chemotherapy failure (refractory cases and relapses). An exception to this is acute myeloid leukaemia (AML), where the *MDR1* gene is often expressed at diagnosis. The reason for this expression is probably the presence of Pgp (product of the *MDR1* gene) on early haematopoietic progenitors (CD34+ cells) [1]. The natural function of this protein is probably to protect this pool of stem cells, capable of self renewal, by pumping out natural xenobiotics potentially toxic for this important compartment. The expression of the *MDR1* gene decreases gradually in more differentiated haemopoietic cells, but persists in normal T cells (mainly CD8+), natural killer cells, and, to a lesser extent, B cells [2–4]. In all other cell lineages, no or very low expression is noted.

Therefore, the MDR phenotype due to the expression of the *MDR1* gene could be "disease related", as in AML and probably in adult T cell lymphoma (ATL) [5], or "treatment related", as in ALL, non-Hodgkin's lymphoma (NHL) or multiple myeloma (MM).

The detection of the MDR phenotype and possibly overcoming MDR is clinically important in haematological malignancies. AML has been extensively studied, with a large majority of studies showing an inverse correlation between the level of Pgp expression and the rate of clinical response to chemotherapy. In addition, a good correlation between the amount of chemotherapy received and Pgp expression of the tumour cells has been observed in multiple myeloma (MM). Several non-randomised phase II studies with chemotherapy and MDR modulators in these two diseases have provided encouraging results, but the role of *MDR1* gene overexpression in clinical drug resistance has yet to be proven, even in AML, the most extensively investigated disease. The major problem is the absence of a standardised technique. For all types of disease, major discrepancies in terms of "positivity" have been observed between groups [6–9], whatever the technique used.

Messenger RNA has been quantified using Northern, slot or dot blot analysis, RNAse protection assay, *in situ* hybridisation and RT–PCR. Protein has been detected by Western blot, immunochemistry or flow cytometry with monoclonal antibodies (C219, MRK16, UIC2, 4ES, etc.) The major advantage of studying leukaemia is the possibility of testing isolated and purified leukaemic cells, and using flow cytometry for detecting phenotype and functional MDR (dye or drug uptake and retention, and effect of modulators on these functions). Few comparative analyses have been published. Herzog and colleagues [10], using several cell lines with different levels of resistance, concluded that all techniques could detect low levels of *MDR1* gene expression, but RT–PCR was the most reliable technique. In another study, on freshly established cell lines from ALL, we also found that RT–PCR and *in situ* hybridisation were the two most reliable techniques [11], while the slot blot method gave some false positive results, and immunocytochemistry with MRK16 was subject to false positive and false negative results. Wall and colleagues [12], in chronic lymphocytic leukaemia, observed an important discrepancy between a low percentage of patients positive for *MDR1* mRNA (evaluated by slot blot), and immunocytochemistry or functional tests. A comparison of monoclonal antibodies on cytospun cells showed that MRK16 is more sensitive than C219 and JSB1, and that flow cytometry is more sensitive than immunocytochemistry [13]. In another comparative study using the same antibodies and APAAP immunocytochemistry, JSB1 appeared to be more reliable [14]. Using flow cytometry, identical results were obtained with MRK16, 4E3 and UIC2 [15]. The quantification of Pgp molecules appears to be impossible, because of the absence of saturation of the sites [16].

A comparison of functional tests and *MDR1* expression (protein or mRNA) indicated a good correlation between dye (rhodamine 1,2,3, DiOC2, Fluo-3) efflux and Pgp expression when measured by flow cytometry [15], but daunorubicin uptake was not directly correlated with Pgp measured by Western blot [17] or by mRNA slot blot [18].

The need for standardisation of flow cytometry analysis (monoclonal antibody and dye/drug efflux), immunocytochemistry and RT–PCR is obvious when results of the same leukaemic samples were analysed by different investigators (manuscript in preparation).

For these reasons, all the data presented here could be criticised, particularly if only immunocytochemistry with one antibody has been used. Since 1994, many publications

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reported the frequency, the prognostic value or the correlation of *MDR1* gene expression with other parameters in haematological malignancies. This paper attempts to summarise these recent data according to the type of disease studied.

ACUTE MYELOID LEUKAEMIA AND MYELODYSPLASIA

Since 1989, several papers have described the MDR phenotype in AML (reviewed in [6–9]). The large majority of reports found between 30 and 50% of "positive" cases, depending on the technique and the threshold used. This percentage was confirmed with refined techniques described in recent studies [15, 19]. In general, Pgp is more frequently encountered in AML after chemotherapy, mainly in patients refractory to chemotherapy [20]. The promyelocytic subtype (AML3) is devoid of Pgp expression [21–23], and all the other AML subtypes do not present with any particular increase in the MDR phenotype.

Recently, several publications examined the functional role of Pgp: Ross and colleagues [17] tested the enhancement of daunorubicin (DNR) accumulation, retention, and cytotoxicity by modulators (verapamil and cyclosporin A) in 49 untreated AML patients. Cyclosporin A (5 μM/l) enhanced the leukaemic cell DNR uptake and retention in 50% of the patients, and enhanced cell killing in 75%. In the same patients, the detection of Pgp by a Western blot technique was positive in only 3 patients, probably because of the lack of sensitivity of the technique. Leith and colleagues [15] observed a decrease in DiOC2, a dye expelled by Pgp, in 39 of the 60 untreated *de novo* AML tested (65%). This efflux was highly correlated with the Pgp phenotype (MRK16) and with an immature phenotype (CD34+). This correlation was firstly emphasised by Te Boekhorst and colleagues [24] in fresh AML samples, and by Bailly and colleagues [16] in myeloid CD34+ cell lines. This correlation between CD34 antigen and Pgp expression has also been noted by Campos and colleagues [25], but not by others [20, 26, 27]. One hypothesis is that the Pgp is functional only in leukaemic cells with an immature CD34+ phenotype. For this reason, dye efflux appears more informative than quantification of Pgp in AML. Another hypothesis is the presence, in CD34+ cells, of another efflux pump, modulated—at least partly—by the same compounds as Pgp.

The MDR phenotype is also frequent in myelodysplastic syndrome (MDS). Holmes and colleagues, in 1989 [28] described an elevation of *MDR1* mRNA by slot blot in 7 out of 19 MDS, but the type of cells expressing the gene was not determined. Later, several groups, using double labelling, showed that the immature haematopoietic cells (CD34+ blast cells) expressed the MDR phenotype [29–31].

MDR1 overexpression in blast crisis of chronic myeloid leukaemia, a secondary leukaemia particularly refractory to chemotherapy, has been reviewed by Pasman and Schouten [6], but no difference was found from that observed in *de novo* AML (36% of overexpression before treatment; 44% after treatment).

The correlation between *MDR1* gene expression and treatment outcome is well-documented in AML [32]. Many studies have reported a relationship between the absence of remission (refractory disease and death during aplasia) and the overexpression of Pgp [24–27, 33–39], or the overexpression of Pgp and refractory disease [20, 40]. Only this latter correlation is a clue of the responsiveness of Pgp in clinical resistance. In several publications, as well as in our own experience, MDR phenotype is also linked with an increase in early deaths [35, 38, 39] during treatment. Overall survival is also often highly correlated with MDR phenotype, but very few reports have shown a correlation between Pgp expression and shorter remission [36, 41], and two groups have not found any correlation between MDR phenotype and response to treatment [42, 43].

It is quite difficult to make definitive conclusions from all these data, concerning the exact role of MDR phenotype in treatment failure: if the drug efflux from leukaemic cells could be responsible for clinical drug resistance, the early deaths, frequently observed in MDR(+) patients, and usually correlated with a poor clinical status, an older age, and toxicity due to the cytostatic treatment, are more difficult to explain.

A phenotype of drug resistance, observed with *MDR1* gene overexpression, could also be due to another efflux pump, for example, the last to be described is MRP, a 190 kD ABC protein [44]. Some discordant results have been published concerning the incidence of *MRP* expression in AML. For Abbaszadegan and colleagues, the leukaemic cells expressed a basal level of the gene [45], but in two recent studies, MRP was found elevated only in relapse [46, 47]. In our group, and in that of Nooter and colleagues, a substantial proportion of untreated cases had elevated *MRP* mRNA (24 and 28%, respectively) [40, 48]. In our study, *MDR1* and *MRP* genes were often co-expressed, and both were predictive for clinical drug resistance. The frequency of co-expression of the two genes preclude conclusions concerning the prognostic value of the two genes in AML, until a sufficient number of patients becomes available for multivariate analysis.

The resistance to cytosine arabinoside, widely used in treatment of AML, has also to be considered for prediction of clinical response. Delmer and coworkers, using multivariate analysis, showed that *in vitro* sensitivity of clonogenic leukaemic cells to the combination of cytosine arabinoside and daunorubicin was one of the best prognostic factors in untreated AML [49]. More recently, Schuurhuis and colleagues showed that the presence of the MDR phenotype with sensitivity to cytosine arabinoside increased the prediction of clinical response in AML [50]. Other potential mechanisms of multidrug resistance have recently been described in AML, such as *BCL2* expression [51], possibly responsible for a decrease in apoptosis following chemotherapy.

ACUTE LYMPHOBLASTIC LEUKAEMIA

Except in a few studies, one using flow cytometry with UIC2 and RT-PCR [52], and the other using immunocytochemistry with JSB1 and C219 [53], or C219 alone [54], the incidence of *MDR 1* overexpression in untreated patients with ALL was low (<10%) at diagnosis [55, 56], and at relapse [57–61], except during the final stage of the disease, when a clinical drug resistance was usually observed [62].

The MDR phenotype is expressed only in poor prognostic subgroups of ALL, that is, adult ALL [53], often Ph1(+), and a subtype of CD7+/CD4−/CD8− ALL [52], which is thought to originate from a lymphohaematopoietic stem cell. In a series of freshly established continuous cell lines derived from high risk childhood ALL, we observed a high incidence of MDR phenotype [11]: the selection—or induction—of a minority of resistant cells during *in vitro* culture could mimic the effect of chemotherapy, and explain the discrepancies

observed in other studies between untreated and heavily treated patients.

The MDR phenotype has not been shown to be predictive for induction treatment failure, except in the Goasguen and coworkers study in adult ALL [53]: the major drug in ALL is a corticosteroid, and not an anthracycline or vincristine [63]. In the same study, the predictive value of Pgp for event-free survival was borderline in childhood ALL ($P = 0.05$), and significant in adults, but the cohort was rather small (23 cases). In a recent multivariate analysis, testing the risk of relapse in 104 childhood ALL, Pgp expression was of borderline significance ($P = 0.07$), far behind the WBC count [54].

It seems reasonable to conclude that *MDR1* overexpression is not often expressed in untreated/first relapsed ALL, except in some poor risk ALL, but is often increased at a final stage, affecting wide clinical drug resistance observed.

MULTIPLE MYELOMA (MM)

Limited bone marrow infiltration by plasma cells (15–60%) in MM supports the use of immunocytochemistry as the method of choice for determining the MDR phenotype in this disease [64]. An alternative technique is double labelling with CD56 or CD38 antibodies together with another antibody against Pgp [65]. As in acute leukaemia, conflicting results have been published, from 0% [65] to 60% [66] of positive cases at diagnosis. Two important series reported a low incidence at diagnosis: Grogan and colleagues [67] and Sonneveld [68] both noted 6% of "positive" cases before treatment. This proportion increased during progression, depending on the dose of anthracyclines and vinca-alkaloids received, reaching 85% in VAD (vincristine, doxorubicin, dexamethasone) refractory patients [68]. This positivity is a factor of clinical resistance to VAD [69]. For this reason, multiple myeloma is a model of drug-induced MDR phenotype, and is a disease of choice for testing the modifier agents associated with the VAD regimen which includes vincristine and doxorubicin, two drugs expelled by Pgp. The plasma cells resistant to treatment with VAD and cyclosporin A are often Pgp negative [70], raising the probability of other mechanisms of drug resistance, such as GSTπ expression [66], or LRP [71].

CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

In CLL, several authors have described a large majority of patients expressing Pgp, either before or after treatment (reviewed in [9]). The semiquantitative studies have shown that the expression is moderate, and does not change after chemotherapy [72]. Holmes and coworkers [73] reported a transient elevation of *MDR1* mRNA in CLL cells during treatment with chlorambucil in 3 patients, raising the question of gene induction, as observed *in vitro*. The MDR phenotype is also detected in normal B lymphocytes [3, 9], and is probably constitutive in CLL, but expressed at a low level, and does not seem to be implicated in clinical drug resistance.

NON-HODGKIN'S LYMPHOMA (NHL)

The majority of studies published in the literature have been retrospective, using immunohistochemical detection of Pgp on frozen lymph node sections (reviewed in [74, 75]). This is the method of choice, routinely performed, and considered suitable for the detection of most membrane glycoproteins in tissue samples. The use of mRNA measurement has been criticised because of the mixture of stroma cells, reactive lymphocytes and tumoral lymphocytes in the same sample. This mixture could give falsely positive results, because of strong positivity of stromal cells and activated macrophages [76]. All the immunostaining methods have used the C219 antibody, and often C219 and JSB1 or MRK16. Between studies, the threshold for positivity varied from a single cell/field to 30% of the tumoral cells, and the proportion of positive samples in untreated patients differed greatly from one study to another. In the majority of publications, samples from treated patients expressed more Pgp than samples from untreated patients. If we assume that mRNA measurements and a low threshold (1%) for positivity are sources of false positive cases, the frequency of MDR phenotype cases seems to be low at diagnosis (2–18%), and higher at relapse (50%). A large study recently published [77] used semiquantitative RT–PCR in 48 patients with relapsed/refractory NHL, before and after polychemotherapy (EPOCH). The proportion of normal and tumoral lymphocytes within the sample was evaluated, and immunoblot for protein quantification confirmed the validity of the mRNA assay. The authors concluded that the contamination of the sample with normal lymphocytes did not greatly modify the results of MDR expression because of the low level of mRNA *MDR1* in these cells. With this sensitive technique, *MDR1* was detected in all samples before EPOCH, and increased by more than 4-fold in 42% of the cases after chemotherapy.

In all the reports, no differences could be observed between high or low grade lymphoma, or between B and T subtypes, except for adult T cell lymphoma (ATL), which frequently expressed the MDR phenotype at presentation [5].

Several studies addressed the question of clinical significance of MDR phenotype in NHL. In a small series, absence of Pgp was associated with a better response [78–80], but in a larger series of high risk lymphoma (57 patients), no correlation with treatment outcome was noted [81].

CONCLUSION

Pgp is often elevated in haematological malignancies, mainly after failure of polychemotherapy. The MDR phenotype as a cause of clinical resistance is documented in AML, myeloma, and possibly in late stages of ALL and non-Hodgkin's lymphoma. The major obstacle for definitive conclusions (20 years after the description of Pgp) is the difficulty in standardising techniques, either for monoclonal antibody or for mRNA quantification.

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Acknowledgements—This work was supported in part by ARC (Grant no. 6078) and Assistance Publique (Grant no. 911508).