

**EXPRESSION OF THE MULTIDRUG RESISTANCE-ASSOCIATED P-GLYCOPROTEIN (P-170)
IN ACUTE LYMPHOBLASTIC LEUKEMIA**

To the Editor:

Recently, Goasguen et al¹ have reported that P-170 was expressed in 38% of 59 cases of de novo acute lymphoblastic leukemia (ALL).

Moreover, they have found that the corresponding multidrug resistance (MDR)-positive phenotype was associated with a higher relapse rate and a shorter remission duration.

We have studied the P-170 expression in leukemic cells of 51

patients with newly diagnosed ALL, including 35 children (median age, 5.5 years) and 16 adults (median age, 31 years). The distribution of immunologic subclasses was 9 T-ALL, 39 pre-pre-B and pre-B-ALL, and 3 biphenotypic ALL.

For the detection of the P-170, we used an immunocytochemical alkaline phosphatase antialkaline phosphatase technique with the monoclonal antibody (MoAb) C219 (CIS International Centocor). Our method differs from that of Goasguen et al¹ by the following reactions: ethanol-methanol or acetone/methanol fixation for 90 seconds at -20°C, incubation with bovine serum albumin or human serum, diluted MoAb (1/20) fixation duration of 30 minutes or overnight.

Two of the 51 patients having more than 1% positive cells were found to be MDR positive at diagnosis. These two patients did not achieve complete remission at the end of the induction treatment (EORTC 58881 protocol for children and EORTC 6861 protocol for adults). This low frequency of the P-170 expression in newly diagnosed ALL has already been reported in other studies.^{2,3}

In the MDR-negative population (49 cases), the complete remission rate was 94% and 75% for children and adults, respectively.

In our series, 7 patients were studied iteratively. Four patients who had relapsed (3 children and 1 adult) remained negative for P-170. Among the 3 other refractory patients (2 children and 1 adult) tested after induction treatment failure, blast cells remained negative in 1, but the percentage of P-170-positive cells increased in the 2 other patients (0% to 5% and 3% to 85%, respectively).

Our data confirm that P-170 expression may be a poor prognostic factor in ALL, but the low incidence of positive patients (4%) observed in our series contrasts with the data reported by Goasguen et al.¹ This kind of discrepancy in MDR phenotype frequency for the same tumor type was frequently observed in the literature.⁴ This

clearly suggests the need for the standardization of different techniques used by people involved in *mdr 1* gene expression measurement.

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RESPONSE

Fenneteau et al report a lower incidence (4%) of P-170 expression than we found (38%) in a previously published study of de novo ALL.¹ Some comments are necessary to explain that difference, one of which concerns the technical procedure. Grogan et al² and Pileri et al³ recommend the use of acetone fixation for P-170 detection by immunocytochemical methods. We have tested fixation with acetone alone, with ethanol/methanol, and with formaldehyde, and our results confirm that acetone fixation provides the best results. The Fenneteau study used instead fixation by ethanol/methanol. This point alone is sufficient to explain the differences in results. Because JSB1 detects an intracytoplasmic epitope of the P-170 protein, the use of a methodology permitting access to an internal antigen without modification or destruction of this antigen is necessary. There are also technical differences in the use of nonspecific incubation (bovine serum albumin) and antibody incubation but these differences are probably not insufficient to explain the low percentage of positive cases in their study. Also, in our series, all antibody incubations were for 1 hour at 4°C, whereas Fenneteau et al used two incubation times (30 minutes and overnight).

Fenneteau et al cite two published studies^{4,5} to support their view. In Ito et al,⁴ only 5 ALL patients were studied. Among these 5 ALL patients, 3 were tested at diagnosis and showed 3.7%, 6.1%, and 5.3%, respectively, P-170-positive cells (tested with MRK16 antibody and flow cytometry). Ito et al⁴ arbitrarily fixed the background level at 5% positive cells. The result should therefore be 1 negative and 2 positive cases. We do not feel that Ito et al⁴ can be used to

support the findings of Fenneteau et al because of the differences in antibody use, methodology, and number of patients.

O'Meara et al⁵ also have a difference in terms of results. O'Meara et al⁵ tested only 10 patients at diagnosis. Only 1 patient was considered to be positive, ie, showing "more than 50% positive cells." This definition does not conform with that used in other studies using a similar APAAP procedure. Also, we consider the number of patients studied to be insufficient for drawing a definitive conclusion.

We can only strongly agree with the proposition of Fenneteau et al that detection of P-170 protein be standardized so that valuable comparisons may be made from study to study.

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