To the Editor:

Recently, two articles of the journal discussed the clinical significance of P-glycoprotein (P-gp)-mediated multidrug resistance (MDR) in human malignancies. As stated in the Editorial, thus far there has been no agreement on standardized screening method for the detection of MDR expressing tumor cells. Techniques determining the level of MDR1 mRNA or immunohistochemical staining of P-gp have been widely used to examine MDR expression in human malignancies. Because posttranslational phosphorylation of P-gp may influence its function, we think that there is still a demand for investigational methods that give information concerning the "pumping" activity of P-gp.

We investigated the P-gp expression in acute leukemia by means of a functional assay. Based on the fluorescent properties of the dye rhodamine 123 (Rh123), which is transported by P-gp, we measured the efflux/retention of this drug by flow cytometry in 29 patients with acute myeloid (AML) and 19 patients with acute lymphoblastic leukemia (ALL). Investigations were performed at time of diagnosis. For dual-fluorescence analysis with Rh123 the leukemic cells were stained with the following phycoerythrin (PE)-conjugated monoclonal antibodies (MoAbs): CD34/CD13/CD33 (AML) and CD10/CD19/CD7 (ALL). Fluorescence gates were placed around the PE-labeled blast cells and Rh123 efflux was selectively assessed in these cells in the presence or absence of 10 μmol/L verapamil.

Seventeen of 29 (59%) AML cases showed a significant Rh123 efflux that was completely blocked in the presence of verapamil (Fig 1). On the contrary, we could detect Rh123 efflux, and thus, P-gp "pumping" activity only in 1 (5%) of 19 ALL patients analyzed. This surprising observation clearly contradicts the results currently published by Goasguen et al. who found 38% P-gp expression in ALL. In this study P-gp expression was determined by immunocytochemistry using two MoAbs (C219 and JSB1). In our ALL series the immunologic subtype of the single Rh123-positive case was of B-ALL. Until now we evaluated four ALL samples (including the Rh123-positive and three Rh123-negative samples) for the expression of MDR1 mRNA by quantitative polymerase chain reaction (PCR). All four cases—Rh123-positive as well as Rh123-negative—expressed MDR1 mRNA at quite the same level. The MDR1 mRNA levels were quantified to the β2-microglobulin amplification product by means of high-performance liquid chromatography.

Although we are aware that our results are preliminary, we want to point out that the detection of P-gp expression by immunocytochemistry or at the mRNA level does not necessarily mean that P-gp is functionally active. Posttranslational modification of P-gp, as described in tissue cultures, may also alter its function in clinical
samples and could explain the discrepancy between the findings of Goasguen et al\textsuperscript{2} and our results with the Rh123 efflux assay in ALL. Consequently, we suppose that investigations which intend to define the clinical role of MDR expression in human malignancies should include MDR screening techniques that provide information about the "pumping" activity of P-gp.

Christof Ludescher
Wolfgang Hilbe
Wolfgang Eisterer
Josef Thaler
Department of Internal Medicine
Markus Gotwald
Johann Hofmann
Institute of Medical Chemistry and Biochemistry
University of Innsbruck
Innsbruck, Austria

REFERENCES
RESPONSE

It is clear that detection of P-170 on the cell membrane, or the mRNA level does not necessarily mean that P-glycoprotein (P-gp) is functionally active as stated by Ludescher et al in the previous letter. Two important comments can be made with respect to their remarks. Firstly, Ludescher et al showed the P-170 (so-called multidrug resistance [MDR]) phenotype in only four acute lymphoblastic leukemia (ALL) cases (all positive) by using a molecular biology technique that cannot be compared strictly with the immunocytochemical staining that we used. It is clear that a standardized technique is necessary. Many previously published reports have addressed this subject. Moreover, we chose this test in view of its easiness and rapidity in a standard laboratory, and because its reliability given that fixation, incubation, antibody specificity, and secondary staining (APAAP technique) are well defined.

Secondly, in our series, we showed that 6 of the 21 P-170-positive cases had less than 11% positive cells; therefore, we can easily suppose that cases with such low number of P-170-positive cells cannot demonstrate Rh 123 efflux by flow cytometry. For the same reason, cases with few P-170-positive cells cannot be detected by flow cytometry, even when using appropriate antibodies. These cases (with low number of P-170-positive cells) have been reported to be resistant cases or cases with early relapse by Musto et al. In our series, two of the six P-170-positive cases (with <11% positive cells) did not achieve complete remission, and three of the four other had a relapse. This shows the high prognostic value of this methodology. Chaudhary and Roninson have demonstrated a strong inverse correlation between the level of P-gp expression and the retention of fluorescent dyes in lymphoid cells, but in their study P-gp was detected in approximately 55% to 65% of the lymphoid cells and in a “large” fraction of the CD34+ cells. These results are not inconsistent with our interpretation. In oncology, prognostic criteria of value are those that predict complete remission and relapse.

However, we agree with Ludescher et al in that posttranslational modification of P-170 can occur in leukemic cells, modifying the role of the transmembrane protein. This point is now under investigation in our laboratory.

Jean E. Goasguen
Beatrice Ly Sunaram
Jean-Marc Dossot
Erwan Mordelet
Renee Fauchet
Laboratoire d’Hématologie
Hopital SUD
Rennes, France

REFERENCES

Low incidence of activity of P-glycoprotein (P-170) in de novo acute lymphoblastic leukemia determined by a flow cytometric assay [letter; comment]

C Ludescher, W Hilbe, W Eisterer, J Thaler, M Gotwald and J Hofmann