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Letters

Expression of *mdr*1 and *mdr*3 Multidrug-Resistance Genes in Hairy Cell Leukaemia

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CHEMOTHERAPY REMAINS an important treatment modality for cytoreduction of leukaemias. However, drug effectiveness can be greatly reduced as a result of the occurrence of cellular drug resistance. Some of the most widely used cytotoxic drugs (i.e. anthracyclines, vinca alkaloids, podophyllotoxins) have *in vitro* been linked to the so-called multidrug-resistance (MDR) phenotype. MDR cells are characterised by a lowered intracellular drug accumulation that is due to activity of an energy dependent unidirectional drug efflux pump with broad substrate specificity [1]. This drug pump is composed of a transmembrane glycoprotein (P-glycoprotein) which is encoded by the human *mdr*1 gene [2]. In man, a second highly homologous gene has been found, which is named alternatively *mdr*2 [3], or *mdr*3 [4].

The *mdr*1 gene has been reported to be expressed in human cancers, including a variety of haematological malignancies [5–7]. Expression of the *mdr*3 gene is mainly found in normal liver [4]. Recently, we found *mdr*3 expression in leukaemia samples. These studies suggested that *mdr*3 expression is exclusively found in B-cell type leukaemias [7, 8]. In the present study, we further investigated the expression of both *mdr*1 and *mdr*3 in the peripheral blood or spleen cells from adult patients with B-hairy cell leukaemia (HCL). A highly specific and sensitive RNase protection assay was used, with a lower limit of detection of approximately 0.2 units [7]. Levels of *mdr*1 and *mdr*3 expression found in peripheral blood or spleen cells from 8 patients with HCL are given in Table 1. Expression of both *mdr*1 and *mdr*3 was detected in all 8 samples studied. In general, *mdr*3 levels were higher than *mdr*1 levels. For the 8 samples studied, the mean level of expression of *mdr*1 and *mdr*3 were 8 U and 22 U, respectively. The *mdr*1 expression levels varied between 0.3 and 35 units (U); the *mdr*3 levels between 3 and 50 U, with no apparent correlation between the two.

Table 1. Expression of mdr1 and mdr3 multidrug-resistance genes in hairy cell leukemia

Patient no.	Expression (units) *mdr*1	mdr3
1	0.3	15
2	0.5	50
3	1	9
4	2	10
5	4	3
6	9	20
7	11	50
8	35	15

Expression levels were determined using an RNase protection assay [7]. The levels were quantitated relative to the expression of KB-8-5 MDR cell line, as proposed in [5].

Treatment of HCL is usually started with splenectomy, followed by prolonged interferon-α, and/or deoxycoformycin administration. Prolonged, control of disease is possible following this scheme [9, 10]. Intensive chemotherapy with cytotoxic drugs, such as anthracyclines, is considered as third line therapy [10]. The results presented in this study show low to intermediate expression of the *mdr*1 gene (related to the classical MDR phenotype) and moderate to high expression of the *mdr*3 gene (presumably also involved in drug resistance [7, 8]) in all studied HCL samples. Therefore, it may be anticipated that results of chemotherapy with MDR-related drugs in HCL patients will be disappointing due to the frequent occurrence of the MDR phenotype. Combination of MDR-related drugs with MDR reversal agents (such as verapamil, cyclosporin and many others) are now evaluated in clinical trials for improved treatment of acute myelocytic leukaemia and multiple myeloma [11, 12]. In these malignancies, elevated levels of *mdr*1 expression have been found, and the acquisition of clinical drug resistance is frequently encountered [6, 7, 11]. When proven successful, such a combination therapy might be a good alternative for third line therapy of hairy cell leukaemia.

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Lymph-node Irradiation in Operable Breast Cancer and Statistical Power

Rodrigo Arriagada and Lars-Erik Rutqvist

PROFESSOR Veronesi *et al.* (vol. 26, pp. 668–670) recently updated results of the Milan breast conservation trial. Patients with positive axillary nodes were included in a second randomisation during the first 3 years of the trial to evaluate the effect of adjuvant radiotherapy to supraclavicular and internal mammary nodes. Results did not show improved survival of treated compared with non-treated patients. Veronesi *et al.* did not state the number of randomised patients. However, if we assume that accrual was constant during the period of patients' entry (88 per year) and that all node positive patients were included (26%), we have estimated that approximately 70 patients may have been studied. If lymph-node irradiation can improve long-term survival by 10% (similar to other adjuvant treatments) the number of patients needed to test this hypothesis is more than 700 (α risk = 0.05, β risk = 0.10). A trial of this size would have a statistical power of 90%—i.e. only a 1:10 chance of concluding that there is no difference when actually there is. The second randomisation of the Milan trial has a statistical power of only 10%—i.e. a 9:10 chance of concluding that there is no difference when really there is. Therefore, the trial is too small to give a definite answer to the question raised.

There is retrospective [1] and prospective [2, 3] evidence that megavoltage lymph-node irradiation with adequate doses can decrease the distant metastasis rate, and consequently may have an effect on overall survival in node positive patients. Because of low statistical power, the Milan trial does not add much knowledge to this issue. Unfortunately, a quick reading of the Milan paper might result in conclusions that are not supported by reported data [4].

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Reply by U. Veronesi *et al.*

THE TRIAL was designed to compare Halsted mastectomy and quadrantectomy plus radiotherapy (QUART) in terms of relapse-free and overall survival.

In our analysis we focused on overall survival, regardless of adjuvant therapies. 4 subgroups have been identified and the log rank test has been carried out accordingly (Table 1).

We agree with Dr Arriagada and Dr Rutqvist that in our paper the sentence concerning radiotherapy might be confusing. However, 16/35 unfavourable events have been observed in subset B radiotherapy and 20/33 in subset C adjuvant radiotherapy; these findings are at the basis of our statement to which, however, no statistical relevance was attributed in the paper.

Table 1.

	A N−	B N+ no adjuvant	C N+ +adjuvant regional RT	D N+ + adjuvant CT
Halsted	263	15	15	56
QUART	257	20	18	57
Total	520	35	33	113

N− = node negative; N+ = node positive; RT = radiotherapy; CT = chemotherapy.

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