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## *In vitro* characterization of drug resistance by means of digital cell image analysis of Feulgen-stained nuclei

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The goal of our work is to propose an original method for detecting the chemoresistance in tumors. We therefore submitted three sensitive and three chemoresistant cell lines to eight anti-neoplastic drugs possessing several mechanisms of action (two alkylating agents, i.e. melphalan and the investigational PE1001; two anti-metabolites, i.e. 5-fluorouracile and hydroxycarbamide; one anthracycline, i.e. doxorubicin; one glycopeptide antibiotic, i.e. bleomycin; one *Vinca* alkaloid, i.e. vinorelbine; and one epipodophyllotoxine derivative, i.e. etoposide). The effects of the treatments were monitored by means of the digital cell image analysis of Feulgen-stained nuclei. This method made it possible to calculate 15 morphonuclear parameters quantitatively describing the morphometric, densitometric and textural features of the cell nuclei. It also enabled the cell cycle kinetics to be determined. The results showed that, except in the case of the vinorelbine, the efficiency of the chemotherapy was statistically correlated with the developmental values of 14 of the 15 parameters. Of these 14 parameters, the most marked correlation appeared with respect to the development of the nuclear size value. In the case of the vinorelbine, the results show that the proportion of mitotic figures in the three chemoresistant cell lines was markedly higher than in the three corresponding chemosensitive ones. In conclusion, the present work shows that the efficiency of chemotherapeutic treatment may be evaluated by means of the digital cell image analysis of Feulgen-stained nuclei. This method could be applied both to hematological and to solid tumors, for which cytopunctures can be carried out serially.

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## Quantitative PCR of drug resistance (DR) related genes in cell lines and in blood and bone marrow from healthy donors

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Many cancers do not respond to chemotherapy (intrinsic resistance) or develop secondary resistance. Such DR phenotypes have been associated with the alteration of gene expression, and the observation that P-glycoprotein (P-gp) mediated DR can be reversed by various agents. This has led to emphasis on work on the molecular and generic mechanisms underlying the DR phenomenon with the hope of pharmacologically modulating the DR phenotype. This paper discussed the development and optimization of a quantitative PCR method linked to HPLC analysis for studying the mRNA levels from various putative clinically relevant DR-associated genes, i.e. members of the transporter gene superfamily (MDR1, MRP), genes involved in cell detoxification (glutathione-S-transferase-$\pi$), genes affecting the DNA structure (topoisomerase II$\alpha$ ) and metabolism (thymidine kinase), and a gene controlling apoptosis (Bcl-2). The validity of the mRNA quantification was established using various control cell lines (DOX, KB, H69), and we routinely obtained a reproducibility of results with standard deviations below 25%. The expression of these five DR parameters was rather stable in peripheral and bone marrow mononuclear cells from healthy donors except that the expression of DNA topoisomerase II $\alpha$ was higher in bone marrow cells, in part because of a higher number of marrow cells in the S-phase. The drug resistant subclones of DOX and KB cell lines seemed to only overexpress MDR1 whereas, in addition to the overexpression of MRP, H69 resistant cells downregulated topoisomerase II$\alpha$ and Bcl-2 genes. Quantitative PCR is a method to accurately measure relative amounts of a given mRNA. Various DR parameters are expressed at a rather stable level in mononuclear cells from healthy donors. In human tumor cell lines, drug resistant subclones may overexpress single or multiple DR parameters compared to their sensitive parent cells, and this seems to depend more on cellular factors than on the drug used to select the DR subclones.

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## Discrimination of topoisomerase II isoenzyme–activities and correlation with cellular sensitivity in small cell samples

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A number of clinically most important cytostatic drugs inhibit topoisomerase II (topo II). Two different isoenzymes named topo II alpha and topo II beta and additional post-transcriptional modifications such as phosphorylation and ribosylation have been described. This study examined whether sensitivity of cells correlates with topo II isoenzyme activity. For this purpose a method was developed to discriminate isoenzyme activities in partially purified nuclear extracts by their catalytic optima at high stringency conditions. In all cells from sensitive and resistant cell lines and clinical material such as peripheral blood and bone marrow