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p170-DEPENDENT MULTIDRUG RESISTANCE. RESTORING FULL SENSITIVITY TO IDARUBICIN WITH VERAPAMIL AND CYCLOSPORIN A DERIVATIVES

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ABSTRACT

Background. **Cell sensitivity to anthracyclines and other drugs depends on several factors, including overexpression of a 170Kd transmembrane glycoprotein (P170) that enhances drug efflux from the cells. Since the result of treatment is negatively related to the expression of P170 in leukemia, malignant lymphoma and other tumors, it is important to investigate drugs and methods that can modify multidrug resistance (MDR).**

Materials and Methods. **Using an MTT-microcultured tetrazolium colorimetric method, we assayed sensitivity to daunorubicin (DNR) and to its 4-demethoxy derivative idarubicin (IDA) in two MDR cell lines (CEM VLB and LOVO DX) and in their respective non-MDR parental lines (CEM and LOVO 109), with and without three MDR modifiers, namely the D-isomer of verapamil (DVRP), cyclosporin A (CyA) and the new CyA derivative SDZ PSC 833.**

Results. **We showed that down-modulation of resistance with MDR modifiers was greater for DNR than for IDA in MDR cells. However, we also demonstrated that restoration of full sensitivity could only be achieved for IDA, not for DNR. DVRP and CyA in combination were more effective than either compound alone and could abolish P170-related resistance to IDA at concentrations of 1-2 μM and 1.6 μM, respectively. SDZ PSC 833 alone was even more effective and set MDR to zero at a concentration ranging between 0.8 and 1.6 μM.**

Conclusions. **These data suggest that combinations of IDA and MDR modifiers may improve the results of cancer and leukemia treatment and that they are worth investigating in vivo, with attention to possible effects on drug pharmacokinetics and on normal tissue damage.**

Key words: multidrug resistance, MDR modifiers, idarubicin

In cancer and leukemia the main factor affecting treatment results is the sensitivity of tumor cells to antitumor agents. Furthermore, in acute leukemia although a complete remission can often be achieved with chemotherapy alone, many patients eventually relapse and die from resistant disease, showing that a significant proportion of leukemic cells are or become drug resistant. A 170-Kd transmembrane glycoprotein (p170) coded by the mdr-1 gene on chromosome 7 is responsible for a mechanism of non specific multidrug resistance (MDR).[1-5] This molecule is frequently overexpressed in cancer, in malignant lymphoma and in leukemia, with expression being inversely related to the result of chemotherapy.[6-15] This protein enhances the efflux of several unrelated compounds from the cells, leading to decreased intracellular drug concentration and probably also to altered intracellular drug dis-

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tribution.[4, 16, 17] This effect applies to a number of drugs that are used for first-line treatment of many tumors, such as anthracyclines and antracenedione compounds, vinca alkaloids, epipodophyllin derivatives and actinomycin D. Knowledge of the molecular basis of MDR can be exploited in several ways, including the search for new compounds and the development of derivatives that are less sensitive to the p170-mediated extrusion mechanism,[18-21] the investigation of compounds that interfere with p170 function or compete for it,[22] the inhibition of mdr-1 gene transcription and translation,[23] and mdr-1 gene transfection of normal hemopoietic cells to limit chemotherapy damage.[24] While the last two approaches are still merely experimental, several investigations have already been dedicated to the development of anthracycline derivatives that escape p170 function, and to the selection of compounds that interfere with p170 function or binding. These compounds, which are usually described as MDR reverting or modifying agents, include the calcium channel blocking agent verapamil (VRP)[25-29] and its D-isomer (D-VRP),[30, 31] as well as the immunosuppressive agent cyclosporin A (CyA) and some CyA derivatives.[32-38] In a prior study we showed that idarubicin, a 4-demethoxy derivative of daunorubicin, was more effective than the parent compound against MDR tumor cells.[39] In this study we show that residual resistance to idarubicin can be minimized by MDR modifiers like DVRP and CyA derivatives, at concentrations that can also be reached and maintained *in vivo*.

Materials and methods

Drugs

The anthracyclines employed were daunorubicin (DNR) and 4-demethoxy-daunorubicin (idarubicin, IDA) (purchased from Farmitalia-Carlo Erba, Italy). Both drugs were dissolved in distilled water at 100 μg/mL and aliquots were stored at –20°C. The MDR modifiers studied were the D-isomer of verapamil (DVRP, a gift of Knoll Farmaceutici, Italy), cyclosporin A (CyA) and its derivative SDZ-PSC 833 (a gift of Sandoz, Basel). DVRP was dissolved in methanol. CyA and PSC were dissolved in ethanol. Aliquots were stored at –20°C.

Cell lines

We used the colon adenocarcinoma cell line LOVO 109 and its MDR doxorubicin (DX)-selected subline LOVO DX, and the T-cell acute lymphocytic leukemia cell line CCRF CEM (CEM) and its MDR vinblastine (VLB)-selected subline CEM VLB. LOVO DX and CEM VLB, but not LOVO 109 and CEM, are characterized by mdr-1 gene amplification and by p170 overexpression.[39, 40] In our laboratory the mean fluorescence intensity (MFI), determined as the ratio of the MFI of the sample processed with MRK-16 and of the one processed with the isotypic control, ranged between 25 and 30 for CEM VLB and between 45 and 55 for LOVO DX, while it varied between 2 and 4 for the two parental non-MDR lines. The methods used for studying p170 have been described elsewhere.[39-41] All cell lines were cultured at 37°C in a humidified atmosphere of 5% CO_2 and maintained in exponential growth in RPMI 1640 (Biochem KG) supplemented with 10% heat-inactivated fetal calf serum (Biochem KG), 2 mM glutamine solution, 100 U/mL penicillin and 100 μg/mL streptomycin (Biochem, KG). The medium of the resistant subline LOVO DX was always supplemented with 200 ng/mL of DX, and that of the resistant subline CEM VLB was always supplemented with 300 ng/mL of VLB until three days before the experiments were performed. For all the studies cells were harvested during exponential growth, washed twice in medium and resuspended at the required concentration.

Drug sensitivity assay

Cell growth in the presence or absence of drugs was determined using the MTT-microcultured tetrazolium colorimetric assay of Mosmann[42] with slight modifications, as described elsewhere.[31, 39] Briefly, anthracyclines with or without MDR modifiers were added at the required concentration after 48h of incubation on microplates. Cell growth and growth inhibition were evaluated after a 7-day incuba-

tion in continuous drug exposure at +37°C in a humidified atmosphere containing 5% CO_2. MTT solution was added at 5 mg/mL, and DMSO was used as the MTT formazan-product solvent. Optical density (OD) was read at 540 nM using a microcultured plate reader (LP400 Diagnostic Pasteur). Results were expressed in terms of percentage of growth as compared to the control. Controls were provided by cells plus MDR modifiers solvents, cells plus anthracyclines and MDR modifier solvents, or cells plus MDR modifiers as appropriate. Appropriate background values (culture medium alone or culture medium plus MDR modifiers) were subtracted from each OD.

All experiments were performed at least in triplicate and the results were the mean of at least three values. Standard deviation was always 10% or less. Inhibition dose 50 (ID50), that is to say the drug concentration which inhibited cell growth by 50% with respect to the control, was calculated as the logarithm of the drug concentration at which the best line fitted by eye to the data would cross a surviving fraction of 0.5. The resistance index (RI) was calculated by dividing the ID50 of the MDR line by the ID50 of the respective non-MDR line.

Results

The two MDR lines (CEM VLB and LOVO DX) were many times more resistant to DNR than to IDA (Table 1), showing that IDA partially eluded the mechanism of action of p170.

DVRP (1-10 μM), CyA (0.8-8 μM) and PSC (0.16-8 μM), alone or in combination, had little effect on the growth of all four cell lines, with growth inhibition ranging between 5% and 30% (data not shown). DVRP (1-10 μM), CyA (0.8-8 μM) and PSC (0.16-8 μM) did not increase the sensitivity of parental non-MDR cell lines (CEM and LOVO 109) to either anthracycline (DNR or IDA) (data not shown).

The effect of DVRP, CyA and PSC on the anthracycline sensitivity of the two MDR lines (CEM VLB and LOVO DX) is shown in Table 2, where the ID50 is reported with and without MDR modifiers. In both cell lines resistance to DNR was much more down-modulated than resistance to IDA. Moreover, a dose-response relationship was more evident with DNR than with IDA, and with DVRP more than with CyA or PSC.

Table 3 shows the effect of DVRP, CyA and PSC on the resistance index (RI). Complete elimination of p170-mediated drug resistance would require reduction of RI almost to 1. For DNR, this result was never obtained with DVRP, was obtained only in CEM VLB with high-dose CyA, and was obtained in both lines with PSC. For IDA, a RI close to 1 and even lower than 1 was obtained in both lines and with all three modifiers.

Combining PSC with DVRP or CyA did not produce any detectable increase in sensitivity to DNR or IDA over PSC alone (data not shown). In contrast, the combination of DVRP and CyA at a relatively low concentration (Table 4) led to a significant increase in sensitivity to DNR, with a RI of 1.5 for CEM VLB and of 5.0 for LOVO DX at DVRP 4 μM + CyA 1.6 μM; this effect was even more pronounced regarding

Table 1. MTT Test; inhibition dose 50 (ID50) for the MDR CEM VLB and LOVO DX cell lines and for their respective parental non-MDR CEM and LOVO 109 lines. Both MDR lines were many times more resistant to DNR than to IDA. The resistance index (RI) is the ratio between the ID50 of the MDR line and the ID50 of the respective non-MDR parental line.

	inhibition dose 50 (ng/mL)			inhibition dose 50 (ng/mL)		
	CEM VLB	CEM	RI	LOVO DX	LOVO 109	RI
DNR	220	4.0	55	500	8.0	62.5
IDA	10	2.5	4	20	2.7	7.4

sensitivity to IDA, which showed a RI of 1 for CEM VLB and of 1.8 for LOVO DX at DVRP 1 or 2 μM + CyA 1.6 μM.

A graphic summary of all these data is shown in Figures 1 and 2. Dose-responses to DNR and to IDA are compared without MDR modifiers, with DVRP and CyA at clinically achievable concentrations (2 μM and 1.6 μM respectively), with a combination of DVRP and CyA at the same concentrations, and with PSC 1.6 μM alone. DVRP and CyA alone were effective at these concentrations, but their dose-response curve remained distant from that of the parental non-MDR line, especially with DNR. DVRP and CyA in combination did better, but only PSC moved the dose-response curve to the level of the dose-response curve of the parental non-MDR lines.

Discussion

This study was designed with the purpose of providing data that could help to improve the clinical use of anthracyclines and other MDR-related drugs in tumor and leukemia treatment. We knew that tumor and leukemia cell lines that display a p170-related MDR phenotype were resistant to DNR,[21, 39-41] and we knew that sensitivity to DNR could be increased by contemporary exposure to a number of different MDR modifier compounds, including VRP and DVRP, CyA and the new CyA derivative SDZ PSC 833. We confirmed these observations in this report but we also showed that with DVRP and with CyA alone it was impossible to abolish p170-related resistance, even at high concentrations that cannot be achieved or maintained *in vivo*. To set MDR to zero it is necessary to bring the ID50 of the MDR line back to the ID50 of the parental non-MDR line, that is to say to obtain a RI of one. However, even at the highest DVRP concentration tested (10 μM) the RI of LOVO DX and of CEM VLB were 6.0 and 4.5, respectively, indicating that p170-related resistance was still operative. At the highest CyA concentration tested (8 μM), the RI of LOVO DX was again substantial (5.0). CEM VLB was more sensitive to CyA and a RI of 1.2 could be obtained with CyA 4 μM, but at that concentration CyA would probably be too toxic for clinical use.

We knew that a combination of VRP and CyA would be more effective than either compound alone,[43] and we confirmed this finding, but we also showed that with a combination of DVRP 2 μM (953 ng/mL) plus CyA 1.6 μM (2000 ng/mL) resistance was not yet fully abolished: RI of 5.0 for LOVO DX and of 2.5 for CEM VLB. In contrast, the new CyA derivative SDZ PSC 833 was able to abolish the resistance of both cell lines to DNR at much lower concentrations (between 0.8 and 1.6 μM), and this effect was not increased by contemporary exposure to DVRP (data not shown).

We knew that MDR cells were more sensitive to the 4-demethoxy derivative of DNR, IDA, than to the parent compound,[21, 39] and we confirmed this finding by showing that the RI to

Table 2. MTT test; inhibition dose (ID50) for the MDR CEM VLB and LOVO DX lines without (controls) and with increasing concentrations of DVRP, CyA and PSC. The decrease in the ID50 was relatively greater for DNR than for IDA since IDA alone was much more toxic than DNR alone.

	CEM VLB ID50		*LOVO DX ID50*	
	DNR	*IDA*	*DNR*	*IDA*
controls	220	10	500	20
DVRP				
1 µM	110	6	300	14
2 µM	70	5	200	10
4 µM	40	3	100	7
6 µM	30	3	70	6
10 µM	18	3	45	5
CyA				
0.8 µM	80	4.5	380	12
1.6 µM	20	3.4	100	10
4 µM	5	3.2	60	6
8 µM	< 5	3.0	40	5
PSC				
0.16 µM	90	4.2	25	7.0
0.4 µM	15	3.6	20	4.0
0.8 µM	6	< 2.5	10	< 2.5
1.6 µM	5	< 2.5	8	< 2.5
4 µM	< 5	< 2.5	8	< 2.5
8 µM	< 5	< 2.5	8	< 2.5

Table 3. Resistance index (RI) to DNR and IDA without MDR modifiers (controls) and with each MDR modifier at each tested dose. The RI was calculated by dividing the ID50 of the MDR line by the ID50 of the respective non-MDR parental line. A RI close to 1 indicates that p170-related resistance was overcome or minimized. This effect was obtained with PSC for both lines and with CyA for CEM VLB; however, the RI was always lower with IDA than with DNR.

	CEM VLB		*LOVO DX*	
	DNR	*IDA*	*DNR*	*IDA*
controls	55.0	4.0	62.5	7.4
DVRP				
1 μM	27.5	2.4	37.5	5.2
2 μM	17.5	2.0	25.0	3.7
4 μM	10.0	1.2	12.5	2.6
6 μM	7.5	1.2	8.7	2.2
10 μM	4.5	1.2	6.0	1.8
CyA				
0.8 μM	20.0	1.8	47.5	4.4
1.6 μM	5.0	1.3	12.5	3.7
4 μM	1.2	1.3	7.5	2.2
8 μM	< 1.0	1.2	5.0	1.8
PSC				
0.16 μM	22.5	1.7	3.1	2.6
0.4 μM	3.6	1.4	2.5	1.5
0.8 μM	1.5	< 1.0	1.2	< 1.0
1.6 μM	1.2	< 1.0	1.0	< 1.0
4 μM	< 1.0	< 1.0	1.0	< 1.0

IDA was about 10 times lower than the RI to DNR (Table 1). On that basis, it was obviously harder to decrease residual resistance to IDA further than it was to lower DNR resistance; however, we showed that DVRP and CyA alone at a low concentration (2 μM and 1.6 μM, respectively) were able to improve the already low RI of IDA, and that a combination of DVRP 1 or 2 μM plus CyA 1.6 μM was even more effective, reducing the RI of LOVO DX to 1.8 and that of CEM VLB to 1.0 or less. The maximum effect was obtained again with PSC, which minimized p170-related resistance at 0.4 μM (RI 1.4 to 1.5) and that abolished resistance at 0.8 μM (RI < 1).

The different sensitivity of MDR cells to DNR and to IDA and the effect of VRP were extensively investigated by Berman and McBride,[21] who used CEM VLB cells and evaluated clonogenic growth inhibition and intracellular anthracycline concentration. From their study it appeared that there was little space for further modulation of IDA sensitivity because they tested IDA at a concentration (1000 ng/mL) that was about 100 times higher than the IDA ID50 of CEM VLB cells. We tested IDA over a wider range of lower concentrations (Figure 1) and demonstrated that within that range resistance to IDA could be reduced further on and could even be abolished with MDR modifier

Table 4. Resistance index (RI) to DNR and to IDA with exposure to a combination of CyA and DVRP at low concentrations. RI, which was calculated by dividing the ID50 of the MDR line by the ID50 of respective parental non-MDR line, was reduced more by CyA and DVRP in combination than by either compound alone. Notice that with CyA 1.6 μM plus DVRP 1 or 2 μM, the RI to IDA was decreased to 1 for CEM VLB and to 1.8 for LOVO DX.

	resistance index (RI)					
	CEM VLB			*LOVO DX*		
	control	CyA 0.8 μM	CyA 1.6 μM	control	CyA 0.8 μM	CyA 1.6 μM
DAUNORUBICIN						
control						
DVRP 1 μM	55.0	20.0	5.0	62.5	47.5	12.5
DVRP 2 μM	27.5	11.2	3.7	37.5	11.2	7.5
DVRP 4 μM	17.5	7.5	2.5	25.0	10.0	5.0
IDARUBICIN						
control	4.0	1.8	1.4	7.4	4.4	3.7
DVRP 1 μM	2.4	1.8	1.0	5.2	2.6	1.8
DVRP 2 μM	2.0	< 1.0	< 1.0	3.7	2.2	1.8

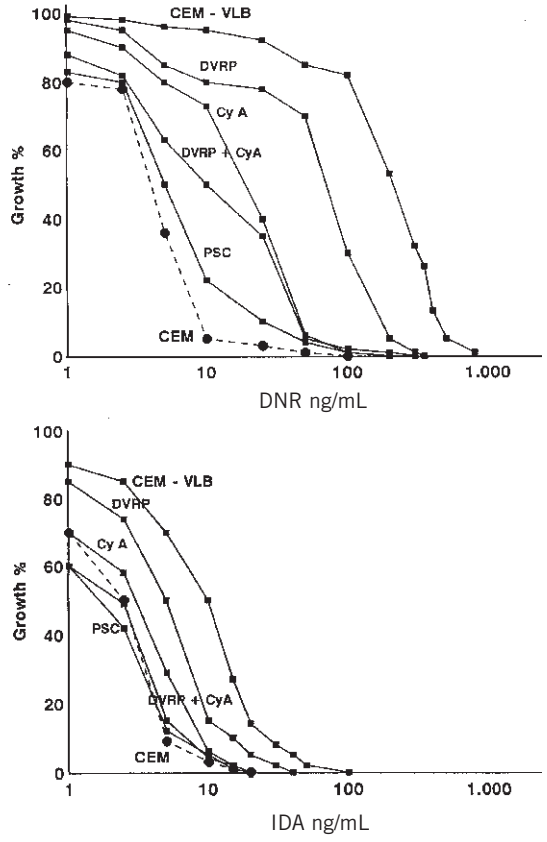

Figure 1. MTT Test; CEM VLB growth inhibition dose-response curve for daunorubicin (DNR) and idarubicin (IDA) with and without low-dose MDR modifiers (DVRP 2 μM, Cya 1.6 μM, DVRP 2 μM+Cya 1.6 μM, and PSC 1.6 μM). The dose-response curve of the non-MDR parental line (CEM) is also shown. The effect of MDR modifiers was greater for DNR than for IDA since IDA alone was more toxic than DNR. However, restoration of full sensitivity was obtained only for IDA with either DVRP+CyA or PSC alone.

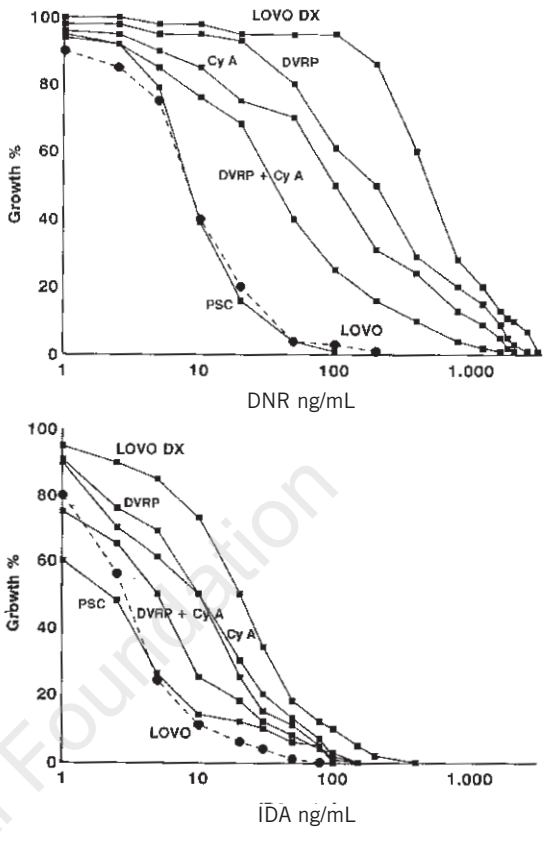

Figure 2. MTT Test; LOVO DX growth inhibition dose-response curve for daunorubicin (DNR) and Idarubicin (IDA) with and without low-dose MDR modifiers (DVRP 2 μM, Cya 1.6 μM, DVRP 2 μM+Cya 1.6 μM, and PSC 1.6 μM). The dose-response curve of the non-MDR parental line (CEM) is also shown. DVRP and CyA in combination were more effective than either agent alone, but they did not restore full sensitivity to either anthracycline. That was obtained only with PSC 1.6 μM.

concentrations that are predictably non toxic and can be tested *in vivo*.

All these data were obtained *in vitro* using two cell systems with different origins (epithelial and lymphocytic), that were characterized by different degrees of mdr-1 gene amplification and of p170 overexpression.[39] CEM VLB, which has fewer mdr-1 gene copies and less p170 than LOVO DX, was less resistant to DNR and IDA, and full sensitivity to either anthracycline could be restored more easily than in LOVO DX. Since the mdr-1 gene is rarely amplified in tumor and leukemic cells[11, 44, 45] and the amount of p170 is usually lower than in *in vitro*-selected MDR cell lines[6, 11, 12] *in vivo* down-modulation of spontaneously occurring MDR could be even more effective than in CEM VLB. MDR down-modulation could also occur in normal cells that constitutively display a MDR phenotype, such as liver, pancreas, kidney, adrenal, colon, and endothelial cells.[4, 7] Moreover, it was shown that normal blood cells also

express p170 and that mdr-1 expression is a feature of normal hemopoietic stem cells.[46-49] Although there is no evidence as yet that p170-directed monoclonal antibodies and immunotoxins are toxic to hemopoietic cells and that MDR modifiers may increase the sensitivity of normal cells to cytotoxic drugs,[50] one would expect that effective adjuvant treatment with MDR modifiers would increase the damage to normal cells, whether hemopoietic or not to some extent. Therefore *in vitro* as well as *in vivo* investigation on that matter is warranted, and or this reason the effect of DVRP and CyA are currently being tested.[22, 28] Nevertheless, we believe that neither compound by itself will possess the necessary efficacy and that the two should be tested in combination. This ought to be possible since their side effects are different. SDZ PSC 833, which proved to be the best *in vitro*, MDR modifier has been reported to be less immunosuppressive and less nephrotoxic than CyA;[33, 36, 37] however, pharmacologic and toxicologic investigation in humans is urgently needed. Another important issue that has not yet been addressed concerns the pharmacokinetic interaction between cytotoxic drugs and MDR modifiers.[51]

In conclusion, the rationale for therapeutic application of MDR modifers is sound, but such application requires information not yet fully available, which must be carefully gathered both *in vitro* and *in vivo*. Since the mechanisms of drug resistance are multiple[1, 52-54] and not only metabolic, but also anatomic and kinetic, it is difficult to predict what the ultimate improvement will be. Nonetheless, it can no longer be ignored that the p170-related MDR phenotype is a significant negative prognostic factor in leukemia, malignant lymphoma and other tumors.

References

1. Pastan I, Gottesman M. Multiple-drug resistance in human cancer. N Engl J Med 1987; 316:1388-93.
2. Piller GJ. Leukemia research fund international research symposium on cytotoxic drug resistance in leukemia and other malignancies. Leukemia 1989; 3:461-7.
3. Carulli G, Petrini M. Multidrug resistance: focus in hematology. Haematologica 1990; 75:363-74.
4. Weinstein RS, Kuszak JR, Kluskens LF, Coon JS. P-glycoproteins in pathology: the multidrug resistance gene family in humans. Hum Pathol 1990; 21:34-48.
5. Baccarani M. Multidrug resistance. Haematologica 1991; 76 (suppl. 3):145-9.
6. Sato H, Preisler H, Day R, Raza A, Larson R, Browman G. MDR1 transcript levels as an indication of resistant disease in acute myelogenous leukaemia. Br J Haematol 1990; 75:340-5.
7. Pileri SA, Sabattini E, Falini B, et al. Immunohistochemical detection of the multidrug transport protein p170 in human normal tissues and malignant lymphomas. Histopathol 1991; 19:131-40.
8. Pirker R, Wallner J, Geissler K, et al. MDR1 gene expression and treatment outcome in acute myeloid leukemia. J Natl Cancer Inst 1991; 83:708-12.
9. Verrelle P, Meissonnier F, Fonck Y, et al. Clinical relevance of immunohistochemical detection of multidrug resistance P-glycoprotein in breast carcinoma. J Natl Cancer Inst 1991; 83:111-6.
10. Campos L, Guyotat D, Archimbaud E, et al. Clinical significance of multidrug resistance P-glycoprotein expression on acute nonlymphoblastic leukemia cells at diagnosis. Blood 1992; 79:473-6.
11. Haber D. Multidrug resistance (mdr-1) in leukemia: is it time to test? Blood 1992; 79:295-8.
12. Michieli M, Damiani D, Geromin A, et al. Overexpression of multidrug resistance-associated p170-glycoprotein in acute non-lymphocytic leukemia. Eur J Haematol 1992; 48:87-92.
13. Zhou DC, Marie JP, Suberville AM, Zittoun R. Relevance of mdr1 gene expression in acute myeloid leukemia and comparison of different diagnostic methods. Leukemia 1992; 6: 879-85.
14. Goasguen JE, Dossot JM, Fardel O, et al. Expression of the multidrug resistance-associated P-glycoprotein (P-170) in 59 cases of de novo acute lymphoblastic leukemia: prognostic implications. Blood 1993; 81:2394-8.
15. Savignano C, Geromin A, Michieli M, et al. The expression of the multidrug resistance related glycoprotein in adult acute lymphoblastic leukemia. Haematologica 1993; 78:261-3.
16. Deuchars KL, Ling V. P-glycoprotein and multidrug resistance in cancer chemotherapy. Semin Oncol 1989; 16:156-9.
17. Endicott JA, Ling V. The biochemistry of P-glycoprotein-mentioned multidrug resistance. Ann Rev Biochem 1989; 58: 137-71.
18. Kaye S, Merry S. Tumor cell resistance to anthracyclines. A review. Cancer Chemother Pharmacol 1985; 14:90-105.
19. Grandi M, Pezzoni G, Ballinari D, et al. Novel anthracycline analogs. Cancer Treat Rev 1990; 17:133-8.
20. Scheulen ME. Development of drug derivatives without cross-resistance to parent compounds. Cancer Treat Rev 1990; 17:67-79.
21. Berman E, McBride M. Comparative cellular pharmacology of Daunorubicin and Idarubicin in human multidrug-resistant leukemia cells. Blood 1992; 79:3267-73.
22. Kaye SB. Reversal of multidrug resistance. Cancer Treat Rev 1990; 17 (Suppl. A):37-43.
23. Scaggiante B, Michelutti A, Damiani D, Michieli MG, Baccarani M, Quadrifoglio F. Formazione della tripla elica in vivo: modulazione dell'espressione genica di MDR1. In: Gambari R, Nastruzzi C, Piva R, eds. Oligonucleotidi sintetici in diagnostica molecolare, 1992; 133-40.
24. Mickisch GH, Aksentijevich I, Schoenlein PV, et al. Transplantation of bone marrow cells from transgenic mice expressing the human MDR1 gene results in long-term protection against the myelosuppressive effect of chemotherapy in mice. Blood 1992; 79:1087-93.
25. Hamada H, Hagiwara KI, Nakajima T, Tsuruo T. Phosphorylation of the Mw 170,000 to 180,000 glycoprotein specific to

multidrug-resistant tumor cells: effects of verapamil, trifluoperazine, and phorbol esters. Cancer Res 1987; 47:2860-5.
26. Yusa K, Tsuruo T. Reversal mechanism of multidrug resistance by verapamil: direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells. Cancer Res 1989; 49:5002-6.
27. Mickisch GH, Kössig J, Keilhauer G, Schlick E, Tschada RK, Alken PM. Effects of calcium antagonists in multidrug resistant primary human renal cell carcinomas. Cancer Res 1990; 50:3670-4.
28. Salmon SE, Dalton WS, Grogan TM, et al. Multidrug-resistant myeloma: laboratory and clinical effects of verapamil as a chemosensitizer. Blood 1991; 78:44-50.
29. Solary E, Bidan JM, Calvo F, et al. P-glycoprotein expression and in vitro reversion of doxorubicin resistance by verapamil in clinical specimens from acute leukemia and myeloma. Leukemia 1991; 5:592-7.
30. Gruber A, Peterson C, Reinzenstein, P. D-verapamil and L-verapamil are equally effective in increasing vincristine accumulation in leukemic cells in vitro. Int J Cancer 1988; 41: 224-6.
31. Damiani D, Michieli M, Michelutti A, Melli C, Cerno M, Baccarani M. D-Verapamil downmodulates p170-associated resistance to doxorubicin, daunorubicin and idarubicin. Anti-Cancer Drugs 1993; 4:173-80.
32. Twentyman PR, Fox NE, White DJG. Cyclosporin A and its analogues as modifiers of adriamycin and vincristine resistance in a multi-drug resistant human lung cancer cell line. Br J Cancer 1987; 56:55-7.
33. Twentyman PR. Modification of cytotoxic drug resistance by non-immuno-suppressive cyclosporines. Br J Cancer 1988; 57: 254-8.
34. Herweijer H, Sonneveld P, Baas F, Nooter K. Expression of mdr1 and mdr3 multidrug-resistance genes in human acute and chronic leukemias and association with stimulation of drug accumulation by cyclosporine. J Natl Cancer Inst 1990; 82:1133-40.
35. Marie JP, Helou C, Thevenin D, Delmer A, Zittoun R. In vitro effect of P-glycoprotein (P-gp) modulators on drug sensitivity of leukemic progenitors (CFU-L) in acute myelogenous leukemia (AML). Exp Hematol 1992; 20:565-8.
36. Boesch D, Muller K, Pourtier-Manzanedo A, Loor F. Restoration of daunomycin retention in multidrug-resistant P388 cells by submicromolar concentrations of SDZ PSC 833, a nonimmunosuppressive cyclosporine derivative. Exp Cell Res 1991; 196:26-32.
37. Keller RP, Altermatt HJ, Nooter K, et al. SDZ PSC 833, a non-immunosuppressive cyclosporine: its potency in overcoming P-glycoprotein-mediated multidrug resistance of murine leukemia. Int J Cancer 1992; 50:593-7.
38. Loor F, Boesch D, Gaveriaux C, Jachez B, Pourtier-Manzanedo A, Emmer G. SDZ 280-446, a novel semi-synthetic cyclopeptolide: in vitro and in vivo circumvention of the P-glycoprotein-mediated tumour cell multidrug resistance. Br J Cancer 1992; 65:11-8.
39. Michieli M, Michelutti A, Damiani D, et al. A comparative analysis of the sensitivity of multidrug resistant (MDR) and non-MDR cells to different anthracycline derivatives. Leuk Lymph 1993; 9:255-64.
40. Gobbi M, Michieli M, Raspadori D, et al. Methods for studying pleiotropic drug resistance (multidrug resistance, MDR). Haematologica 1991; 76 (suppl. 3):150-3.
41. Geromin A, Michieli M, Damiani D, et al. Cancer chemotherapy does not enhance MDR-associated 170 kd glycoprotein expression in normal blood mononuclear cells. Haematologica 1992; 77:470-2.
42. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. J Immunol Methods 1993; 65:55-63.
43. Hu XF, Martin TJ, Bell DR, de Luise M, Zalcberg JR. Combined use of cyclosporin A and verapamil in modulating multidrug resistance in human leukemia cell lines. Cancer Res 1990; 50:2953-7.
44. Holmes J, Jacobs A, Graham C, Janowska-Wieczorek A, Padua RA. Multidrug resistance in haemopoietic cell lines, myelodysplastic syndromes and acute myeloblastic leukaemia. Br J Haematol 1989; 72:40-4.
45. Michieli M, Giacca M, Fanin R, Damiani D, Geromin A, Baccarani M. mdr-1 gene amplification in acute lymphoblastic leukaemia prior to antileukaemic treatment. Br J Haematol 1991; 78:290-1.
46. Chaudhary PM, Roninson IB. Expression and activity of P-glycoprotein, a multidrug efflux pump, in human hematopoietic stem cells. Cell 1991; 66:85-94.
47. Drach D, Zhao S, Drach J, et al. Subpopulations of normal peripheral blood and bone marrow cells express a functional multidrug resistant phenotype. Blood 1992; 80:2729-34.
48. Damiani D, Michieli M, Michelutti A, et al. Expression of multidrug resistance gene (mdr-1) in human normal leukocytes. Haematologica 1993; 78:12-7.
49. Marie JP, Brophy NA, Ehsan MN, et al. Expression of multidrug resistance gene mdr1 mRNA in a subset of normal bone marrow cells. Br J Haematol 1992; 81:145-52
50. Baccarani M, Damiani D, Michelutti A, Michieli M. Expression of multidrug resistance gene (mdr1) in normal hematopoietic cells (correspondence). Blood 1993; 81:3480-1.
51. Tortorice KL, Heim-Duthoy KL, Awni WM, Rao KV, Kasiske BL. The effects of calcium channel blockers on cyclosporine and its metabolites in renal transplant recipients. Therap Drug Monit 1990; 12:321-8.
52. Wolley PV III, Tew KD. Mechanism of drug resistance in neoplastic cells. Bristol-Meyers Cancer Symposia, Academic Press, 1988.
53. Beck WT. Mechanisms of multidrug resistance in human tumor cells. The roles of P-glycoprotein, DNA topoisomerase II, and other factors. Cancer Treat Rev 1990; 17 (suppl. A): 11-20.
54. Holmes JA. Multidrug resistance in leukemia. Leuk Lymph 1990; 1:163-8.