Clinical Reversal of Multidrug Resistance

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ABSTRACT

Reversal of drug resistance offers the hope of increasing the efficacy of conventional chemotherapy. We tested dexverapamil as a P-glycoprotein antagonist in combination with EPOCH chemotherapy in refractory non-Hodgkin’s lymphoma. In a cross-over design, dexverapamil was added to EPOCH after disease stabilization or progression occurred. Objective responses were observed in 10 of 41 assessable patients. Biopsies for \textit{mdr-1} were obtained before EPOCH treatment and at the time of cross-over to dexverapamil. Levels of \textit{mdr-1} were low before EPOCH, but increased fourfold or more in 42% of patients in whom serial samples were obtained. Pharmacokinetic analysis revealed median peak concentrations of dexverapamil and its metabolite, nor-dexverapamil, of 1.66 µmol/l and 1.58 µmol/l, respectively. Since both are comparable antagonists, a median peak total reversing concentration of 3.24 µmol/l was achieved. Pharmacokinetic analysis of doxorubicin and etoposide levels confirmed a delay in the clearance of doxorubicin ranging from 5% to 24%; no change in the pharmacokinetics of etoposide was observed. This study provides sufficient rationale for testing dexverapamil in a randomized clinical trial.

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Drug resistance is a major factor limiting the effectiveness of cancer chemotherapy. This drug resistance takes two forms. One is intrinsic resistance, i.e., resistance which is already present in tumor cells at the time of first treatment. The second type is acquired resistance, i.e., resistance which emerges by adaptation or selection after treatment has been given. Table 1 presents an outline of five pathways by which cells become drug resistant [1, 2]. The first pathway is modulation of the intracellular concentration of drug. This can be done through decreasing drug influx or increasing drug efflux. Currently known drug efflux proteins are P-glycoprotein, which transports paclitaxel, \textit{vinca} alkaloids, anthracyclines and other natural products; and MRP, the multidrug resistance related protein which is associated with resistance to etoposide and adriamycin. A second pathway which a cell employs to become drug resistant is through alteration of the cellular metabolism of a drug, either through decreased activation or increased deactivation. Glutathione conjugation prior to transport out of the cell is an example of a drug detoxification mechanism. A third pathway to drug resistance is alteration of the cellular target of the drug. Examples of this are the mutations which have been shown to render topoisomerase resistant to messenger amsacrine and which are presumed to occur in tubulin, thereby preventing binding of paclitaxel or \textit{vinca} alkaloids to the tubulin. A fourth pathway which a cell may take to become drug resistant is through enhancement of repair, as for repair of DNA damage due to alkylating agents. Finally, a fifth pathway which may promote drug resistance but has not been adequately explored to date is that of the cell survival pathways. These may involve growth receptor pathways, signal transduction pathways and apoptosis pathways.

Thus, P-glycoprotein can be viewed as an integral part of multiple cellular resistance mechanisms. It is a 170 kDa membrane surface glycoprotein which mediates resistance as an energy-dependent drug efflux pump [3]. It is found in normal tissues including the adrenal cortex, the proximal tubules of the kidney, the biliary system, the pancreas, the colon, and the capillary endothelial cells of the vessels in the brain and testes. The role in all of these normal tissues is not well understood; however, knockout of the \textit{mdr-1} homolog in the mouse resulted in delayed drug clearance and increased concentrations of toxins in the brain [4]. Drugs that are transported by
P-glycoprotein constitute a broad spectrum, including doxorubicin, paclitaxel, vinblastine, mitoxantrone, VP-16, and probably hundreds or thousands of other compounds. In addition to compounds which are transported by P-glycoprotein, a number of nontoxic compounds also appear to interact with it and have the property of blocking drug efflux when given in higher concentrations. The central question to be answered is whether reversal or prevention of P-glycoprotein-mediated drug resistance will have a significant clinical impact [5].

P-glycoprotein antagonists have been used in a variety of malignancies in a phase II clinical trial design to attempt reversal of clinical drug resistance. Table 2 highlights a number of the studies [6-15]. All of the antagonists used in these so-called “first generation studies” are FDA-approved for other indications and were found to have P-glycoprotein blockade as one of their features. The majority of the studies in Table 2 were carried out using cyclosporine A, the most potent P-glycoprotein antagonist available at the time. The responses in these trials varied from none to 70%, depending on the malignancies studied. In some studies the chemotherapeutic regimen given in combination with the antagonist was different from that which the patient had previously received, and thus it is not clear that patients were actually refractory to the treatment. In addition, the regimens in some studies included a schedule change, in which the chemotherapy was given over a longer duration than previously, which may itself have resulted in the responses observed [8, 11, 16].

Third, cyclosporine and potentially other P-glycoprotein antagonists are able to delay drug clearance and increase the area under the curve (AUC), which may have increased the dose intensity and the exposure duration [17, 18]. Thus, one cannot be sure of the contribution of the P-glycoprotein antagonist to the responses observed, and these issues point out the difficulties in using a standard phase II design for clinical trials aimed at P-glycoprotein reversal. To avoid these difficulties, a clinical trial was launched at the National Cancer Institute in patients with refractory lymphoma using a cross-over design. These patients received EPOCH chemotherapy until the time when disease progression or stable disease was documented. Subsequently, patients continued to receive EPOCH with the addition of oral dexverapamil, the D-stereoisomer of verapamil (treatment schema Table 3) [19-21]. Initially, a phase I study was carried out. The dose-limiting toxicities for dexverapamil included congestive heart failure, hypotension and heart block, with a maximum tolerated dose of 150 mg/m² every 4 h [22]. Although the intent of the study was to enroll refractory patients, EPOCH salvage chemotherapy itself was surprisingly effective [23]. The combined complete and partial response rate to EPOCH was 100% for

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Malignancy</th>
<th>Antineoplastic agent(s)</th>
<th>Reversal agent</th>
<th>Responses (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presant, 1986</td>
<td>Advanced malignancies</td>
<td>Adriamycin</td>
<td>Verapamil</td>
<td>3/13 (23%)</td>
<td>[6]</td>
</tr>
<tr>
<td>Ozols, 1987</td>
<td>Ovarian cancer</td>
<td>Adriamycin</td>
<td>Verapamil</td>
<td>0/8 (0%)</td>
<td>[7]</td>
</tr>
<tr>
<td>Miller, 1991</td>
<td>Lymphoma</td>
<td>CVAD</td>
<td>Verapamil</td>
<td>13/18 (72%)</td>
<td>[8]</td>
</tr>
<tr>
<td>Isonishi, 1991</td>
<td>Ovarian cancer</td>
<td>VP-16</td>
<td>Dipyridamole</td>
<td>1/16 (6%)</td>
<td>[9]</td>
</tr>
<tr>
<td>Philip, 1992</td>
<td>Advanced malignancies</td>
<td>VP-16</td>
<td>Nifedipine</td>
<td>1/15 (6%)</td>
<td>[10]</td>
</tr>
<tr>
<td>Yahanda, 1992</td>
<td>Advanced malignancies</td>
<td>VP-16</td>
<td>Cyclosporin A</td>
<td>4/57 (6%)</td>
<td>[12]</td>
</tr>
<tr>
<td>Marte, 1993</td>
<td>Acute leukemia</td>
<td>Mitox + VP-16</td>
<td>Cyclosporin A</td>
<td>6/16 (40%)</td>
<td>[13]</td>
</tr>
<tr>
<td>Samuels, 1993</td>
<td>Advanced malignancies</td>
<td>Vinblastine</td>
<td>Cyclosporin A</td>
<td>760MR (11%)</td>
<td>[14]</td>
</tr>
<tr>
<td>List, 1993</td>
<td>Acute leukemia</td>
<td>Dauno + AraC</td>
<td>Cyclosporin A</td>
<td>29/42 (60%)</td>
<td>[15]</td>
</tr>
<tr>
<td>Bates, 1995</td>
<td>Breast cancer</td>
<td>Adriamycin or Vinblastine</td>
<td>Amiodarone</td>
<td>9/33 (27%)</td>
<td>[16]</td>
</tr>
</tbody>
</table>
PCR was carried out as previously described. Dexverapamil. No biopsy was obtained at the time of cross-over. Quantitative prior to EPOCH, and at autopsy, following treatment with EPOCH and several samples in a patient enrolled in the study. Biopsies were obtained whenever possible from patients before and after treatment with EPOCH alone and then following treatment with EPOCH plus dexverapamil. Responses were noted in 10 of 41 assessable patients, including three (7%) with a complete response, two (5%) with a partial response, and five (12%) with a minimal response. Minimal (i.e., less than 50% reduction in tumor size) responses were reported because they represented an objective change in the tumor growth pattern. In order to understand the significance of the responses observed, we examined the role of P-glycoprotein, the role of the antagonist, and the role of pharmacokinetic alterations. We asked whether mdr-1 or P-glycoprotein expression was present. Second, we asked whether verapamil levels were high enough to block P-glycoprotein. Third, we asked whether verapamil altered the pharmacokinetics of doxorubicin or etoposide.

To address the first question, biopsies were obtained whenever possible from patients before and after treatment with EPOCH alone and then following treatment with EPOCH plus dexverapamil. A polymerase chain reaction (PCR) assay was developed and validated in order to quantitate mdr-1 expression in some of the very small patient samples which were obtained as part of the study [24, 25]. Samples were obtained by incisional biopsy or aspiration of abnormal lymph nodes. Total RNA was harvested, cDNA was obtained by reverse transcription and, as previously described, quantitative PCR was carried out. The cDNA was serially diluted and amplification using mdr-1 or β2-microglobulin-specific primers was carried out for 30 cycles. PCR products were run on a gel which was stained with ethidium, photographed and quantified by densitometry. All results were compared to the level of expression in the SW620 control cell line which was included in every experiment. Marked overexpression of P-glycoprotein developed in several patients’ tumors during the course of treatment. Results from serial biopsies in one patient are shown in Figure 1. Pretreatment, mdr-1 expression was detectable in 125 and 250 ng total RNA from two biopsy sites, while following treatment with EPOCH and EPOCH plus dexverapamil and death from disease, expression of mdr-1 is readily detectable in various tumor sites in 15 ng RNA. The calculated mdr-1 levels, normalized to β2-microglobulin, for this patient were 1.8 and 2.7 for the skin and stomach biopsies, respectively, before treatment, and 326, 134, 467, and 412 for the tumors from the omentum, axilla, spleen and mesentery, respectively, following treatment. In 19 patients, serial biopsies were obtained pre- and post-EPOCH chemotherapy (before cross-over). In 42% of patients increases of fourfold or more were noted in mdr-1 expression in the biopsy samples [24]. In 58% of patients, increases in mdr-1 were not observed in the biopsy samples, suggesting that other mechanisms of resistance prevailed. However, the very large increases observed in individual cases strongly suggest that mdr-1 plays a role in clinical drug resistance in a subset of patients. Among 12 patients in whom both serial samples were available and responses to the addition of dexverapamil were evaluable, three patients had a partial or minimal response, while nine patients had progressive disease or no response (Table 4). It is of interest to note that increases in mdr-1 expression of fourfold or more were observed in two of the three patients whose tumors responded to the addition of dexverapamil, while a similar increase was observed in only three of the nine patients whose tumors did not respond. This would imply that a correlation may exist between increases in mdr-1 expression and response to dexverapamil, but the few numbers do not allow a firm conclusion. Further, the data suggest that in some patients, mdr-1 could not be overcome with dexverapamil but that for other patients, mdr-1 did not play a role in clinical drug resistance.

If mdr-1 did play a role in resistance but could not be reversed by dexverapamil, were the blood levels of dexverapamil high enough to achieve reversal? Figure 2 demonstrates that both dexverapamil and its metabolite, nor-dexverapamil, reverse P-glycoprotein-mediated

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**Table 3. EPOCH treatment schema**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosing Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etoposide</td>
<td>50 mg/m²/d CIV d 1-4</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.4 mg/m²/d CIV d 1-4</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>10 mg/m²/d CIV d 1-5</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>750 mg/m² IV d 6</td>
</tr>
<tr>
<td>Prednisone</td>
<td>60 mg/m²/d PO d 1-6</td>
</tr>
<tr>
<td>Dexverapamil</td>
<td>150 mg/m² q4h PO d 0-6</td>
</tr>
</tbody>
</table>

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**Figure 1.** Quantitative polymerase chain reaction (PCR) assay of mdr-1 in several samples in a patient enrolled in the study. Biopsies were obtained prior to EPOCH, and at autopsy, following treatment with EPOCH and dexverapamil. No biopsy was obtained at the time of cross-over. Quantitative PCR was carried out as previously described.
Table 4. Results of mdr-1 measurement in serial samples from 12 patients evaluable for response to EPOCH/dexverapamil

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Pre-EPOCH $^a$</th>
<th>Post-EPOCH $^b$</th>
<th>$\Delta$ $^c$</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>4.70</td>
<td>19.00</td>
<td>$&gt;$</td>
<td>PR</td>
</tr>
<tr>
<td>02</td>
<td>1.40</td>
<td>0.45</td>
<td>$&lt;$</td>
<td>PR</td>
</tr>
<tr>
<td>03</td>
<td>5.90</td>
<td>32.00</td>
<td>$&gt;$</td>
<td>MR</td>
</tr>
<tr>
<td>04</td>
<td>2.90</td>
<td>18.40</td>
<td>$&gt;$</td>
<td>NR</td>
</tr>
<tr>
<td>05</td>
<td>0.10</td>
<td>9.50</td>
<td>$&gt;$</td>
<td>NR</td>
</tr>
<tr>
<td>06</td>
<td>0.16</td>
<td>38.50</td>
<td>$&gt;$</td>
<td>NR</td>
</tr>
<tr>
<td>07</td>
<td>2.50</td>
<td>1.60</td>
<td>$&lt;$</td>
<td>NR</td>
</tr>
<tr>
<td>08</td>
<td>6.00</td>
<td>3.50</td>
<td>$&lt;$</td>
<td>NR</td>
</tr>
<tr>
<td>09</td>
<td>0.46</td>
<td>0.56</td>
<td>$&lt;$</td>
<td>NR</td>
</tr>
<tr>
<td>10</td>
<td>0.95</td>
<td>1.30</td>
<td>$&lt;$</td>
<td>NR</td>
</tr>
<tr>
<td>11</td>
<td>4.40</td>
<td>12.20</td>
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<td>NR</td>
</tr>
<tr>
<td>12</td>
<td>3.00</td>
<td>2.60</td>
<td>$&lt;$</td>
<td>NR</td>
</tr>
</tbody>
</table>

$^a$The mdr-1 levels were reported from PCR analysis following normalization to the expression of a control gene, $\beta_2$ microglobulin [24]. These levels were related to the levels found in the unselected SW620 human colon cancer cell line, which was assigned a value of 10 for its mdr-1 expression.

$^b$Samples were obtained before EPOCH treatment, and at the time of cross-over to treatment with dexverapamil plus EPOCH.

$^c$Changes in mdr-1 level: $>$ indicates a fourfold or greater increase in mdr-1 following EPOCH alone; $<$ indicates less than a fourfold increase.

resistance in an unselected colon cancer cell line, HCT-15, and an unselected renal cell line, UO-31. Both compounds at 6.3 µg/ml increase cytotoxicity from DINIB, a cytotoxic natural product and P-glycoprotein substrate, to a degree comparable to that seen with racemic verapamil [26]. At the maximum tolerated dose, the median

Figure 2. In vitro reversal of P-glycoprotein-mediated resistance by dexverapamil and its metabolite, nor-dexverapamil. Cytotoxicity assays of DINIB, NCS 80467 (4,9-dihydro-3-isobutyl-2-methyl-1-(p-nitrophenyl)-4,9-dioxo-1H-naph[2,3-d]imidazolium bromide), a P-glycoprotein substrate used to assay the potential of a P-glycoprotein antagonist to enhance cytotoxicity [26]. Cells were cultured in 96 plates and treated for 48 h before protein content was determined, as previously described [27, 28].
steady-state levels were 1.21 \( \mu \text{mol/l} \) for dexverapamil and 1.43 \( \mu \text{mol/l} \) for nor-dexverapamil, its metabolite [22]. Median peak plasma levels in this cohort of patients were 1.66 \( \mu \text{mol/l} \) and 1.58 \( \mu \text{mol/l} \) for dexverapamil and its metabolite, respectively. Since these are comparable blockers, one can conclude that the median steady-state “blocking” level was 2.64 \( \mu \text{mol/l} \), and the median peak plasma “blocking” level was 3.24 \( \mu \text{mol/l} \), concentrations approaching those used in the laboratory to antagonize low levels of P-glycoprotein in multidrug resistant cell lines but insufficient to antagonize high levels [20, 21, 29]. We compared responses in the patients to the level of verapamil measured in the plasma and found no correlation between response and dexverapamil level.

To evaluate whether there was a pharmacokinetic impact by dexverapamil, steady-state concentrations of doxorubicin and etoposide were obtained in seven patients in the cycle immediately before and the first cycle with verapamil [22]. Levels of etoposide were stable before and after the addition of dexverapamil, while there was an increase in the doxorubicin steady-state concentration which ranged from 5% to 224% in seven patients. This suggests that there was an impact of dexverapamil on the elimination of doxorubicin. Whether this could have impacted on the disease response observed is not known.

**DISCUSSION**

In summary, in a cross-over design, EPOCH chemotherapy was given to patients with refractory lymphoma. The D-stereoisomer of verapamil was added to EPOCH as a P-glycoprotein antagonist at the time of disease progression. The purpose of this trial was to determine whether reversal of P-glycoprotein would impact significantly on the treatment of refractory lymphoma. One hundred and one patients with non-Hodgkin’s lymphoma were enrolled on EPOCH alone; this was found to be a salvage regimen which merited further attention in the clinic. The addition of dexverapamil in 49 patients yielded a 12% objective response rate including complete and partial responses. Increased mdr-1 levels following EPOCH were found in 42% of serial samples, suggesting that mdr-1 plays a role in resistance in a subset of patients with lymphoma [24]. The median steady-state concentration of dexverapamil plus its metabolite was 2.64 \( \mu \text{mol/l} \) at the maximum tolerated dose. Interpretation of the data is confounded by small increases in the steady-state concentration of doxorubicin following the addition of dexverapamil.

This trial has amply illustrated the difficulty of performing P-glycoprotein reversal studies in the clinic. A cross-over design was used in order to gain information about the effect of dexverapamil alone, as described in a previous trial of P-glycoprotein reversal [30]. With this design the advantage is that the patient serves as his/her own control and the assumption is that any response following the addition of dexverapamil (when chemotherapy is maintained in the same schedule and dosage) is due to the addition of the antagonist. This assumption, however, may be flawed. First, verapamil has activities in cells other than blocking P-glycoprotein. It has a dose-response curve and shows cytotoxicity at high concentrations in cells in vitro (admittedly at concentrations which were higher than those achieved in the present study). Secondly, there is the impact on pharmacokinetics. Studies using cyclosporine A as a P-glycoprotein antagonist first demonstrated that a delay in etoposide clearance resulted in increased and prolonged steady-state concentrations of the chemotherapeutic agents [17]. This impact could be mediated either by direct P-glycoprotein blockade in the liver and delay of excretion of chemotherapeutic agents, or by acting through metabolic pathways, with competition for enzymes needed to metabolize both verapamil and doxorubicin. In either case the increase in the AUC results in higher blood levels and blood levels which are present for a longer period of time. Thus, there is an increase in both the intracellular concentration of drug and in the duration to which tumor cells are exposed to the chemotherapy, and these increases in dose intensity could affect response rate. Thus, while the responses observed occur after the addition of verapamil, it is not yet certain that the responses are due to overcoming P-glycoprotein in the clinic.

A second means by which we could determine that P-glycoprotein was being overcome in patients is by comparing responses and P-glycoprotein levels in tumors. This study demonstrated that mdr-1 was readily detected and that the level increased, but that the precise level did not correlate with response. However, the numbers were small, and it is still not possible to conclude whether mdr-1 expression could predict for a response to verapamil. The 58% of patients who had low levels of P-glycoprotein expression that did not change indicated that other mechanism(s) of resistance are present in lymphoma. There was a trend toward higher numbers of patients with low levels in the group that did not respond to verapamil.

The data obtained in this study confirm and extend results obtained at the University of Arizona in which 64% of patients with refractory lymphoma had overexpression of mdr-1 [8]. Interestingly, the biopsies obtained prior to EPOCH alone had uniformly low but detectable mdr-1 expression, while 42% had increased levels following progression on EPOCH. This identifies a third problem with the cross-over design—exposure to a given chemotherapeutic regimen results in increased resistance to that regimen, with both P-glycoprotein and non-P-glycoprotein mechanisms advancing.
These considerations point out the need for more potent P-glycoprotein antagonists which are able to block any level of P-glycoprotein-mediated drug efflux in vitro and, more importantly, for studies of P-glycoprotein antagonists that are able to validate the assumption that intracellular concentrations of chemotherapy are increasing in patients treated with P-glycoprotein antagonists. One approach that has been proposed is to use Tc-99m Sestamibi, a radiopharmaceutical conventionally used in cardiac imaging studies and found to be a substrate for P-glycoprotein-mediated transport [31]. It may be possible to obtain a tumor image and to observe efflux which could be modulated with the antagonist. Other approaches in hematopoietic malignancies may be to measure chemotherapeutic concentrations in tumor cells before and after the addition of the P-glycoprotein antagonist. Alternatively, ex vivo assays may be developed to look at drug efflux in the presence or absence of clinical administration of the antagonist.

Ultimately, it is clear that further advances in chemotherapy will require overcoming several different mechanisms of drug resistance, of which P-glycoprotein is the first to receive extensive clinical testing. Although we have not proven with certainty that verapamil reversed clinical resistance, responses in 10 of 41 patients with refractory non-Hodgkin’s lymphoma in a controlled trial provide evidence for reversal. The data support a randomized trial testing the addition of verapamil or other reversing agent to therapy in lymphoma. Such a trial should be carried out in patients much earlier in their disease course, prior to the development of multiple mechanisms of resistance.

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REFERENCES

19 Wilson WH, Bates SE, Fojo AT et al. Controlled trial of dextra-
larin, a modulator of multidrug resistance, in lymphomas refrac-

20 Plumb JA, Milroy R, Kaye SB. The activity of verapamil as
a resistance modifier in vitro in drug resistant human tumor
cell lines is not stereospecific. Biochem Pharmacol
1990;39:787-792.

21 Mickisch GH, Merlino GT, Aiken PM et al. New potent vera-
pamil derivatives that reverse multidrug resistance in human
renal carcinoma cells and in transgenic mice expressing the

22 Wilson WH, Jamis-Dow C, Bryant G et al. Phase I and phar-
macokinetic study of the multidrug resistance modulator
dexverapamil with EPOCH chemotherapy. J Clin Oncol

23 Wilson WH, Bryant G, Bates SE et al. EPOCH chemother-
apy: toxicity and efficacy in relapsed and refractory

24 Kang YK, Zhan Z, Regis J et al. Expression of mdr-1 in
refractory lymphoma: quantitation by polymerase chain reac-

25 Murphy LD, Herzog CE, Rudick JB et al. Use of the poly-
merase chain reaction in the quantitation of mdr-1 gene

26 Alvarez M, Paul K, Monks A et al. Expression of mdr-1/P-
glycoprotein in the cell lines of the NCI anticancer drug screen
program as a tool to identify novel P-glycoprotein substrates

27 Monks A, Scudiero D, Skehan P et al. Feasibility of a high-
flux anticancer drug screen using a diverse panel of cultured

28 Skehan P, Storeng R, Scudiero D et al. New colorimetric cyto-
toxicity assay for anticancer drug screening. J Natl Canc Inst
1990;82:1107-1112.

29 Lai G-M, Chen Y-N, Mickley LA et al. P-glycoprotein
expression and schedule dependence of adriamycin cytotoxi-
city in human colon carcinoma cell lines. Int J Cancer

30 Salmon SE, Dalton WS, Grogan TM et al. Multidrug-resis-
tant myeloma: laboratory and clinical effects of verapamil as

31 Piwnica-Worms D, Chiu ML, Budding M et al. Functional
imaging of multidrug-resistant P-glycoprotein with an organ-