USA the FDA has approved a trial to be conducted by the NCI/NSABP for high-risk women over the age of 35 years or all women over the age of 60 years. Women will be randomised to receive 5 years tamoxifen (20 mg daily) or placebo (B. Fisher, Pittsburgh).

Improvements in the endocrine therapy of advanced disease are also being attempted by comparing tamoxifen plus a luteinising hormone-releasing hormone (LHRH) agonist (goserelin) versus goserelin alone. Preliminary results suggest an improvement in the duration and survival of responsive patients receiving the combination. Recruitment of 350 patients to a large trial is complete and an analysis will be made in late 1991 (R. Blamey, Nottingham).

Similarly a new drug, ICI 182,780, is about to be evaluated to treat patients who fail long-term adjuvant tamoxifen therapy. The drug is a pure anti-oestrogen in laboratory tests and has now successfully passed the initial toxicology evaluation (A. Wakeling, Macclesfield). The mechanism of action of pure anti-oestrogens seems to be to prevent receptor dimerisation that is required for interaction at the DNA to activate oestrogen-specific genes (M.G. Parker, London). The pure anti-oestrogen (ICI 164,384, a compound related to ICI 182,780) inhibits the growth of tamoxifen stimulated tumours in the laboratory so this new class of drug can potentially be used as a second-line endocrine therapy after tamoxifen (V.C. Jordan, Madison). Clinical evaluation has started in the UK.

Finally, novel new strategic approaches to treat breast cancer were considered. Most disease is hormone-independent so progress to control cell replication would have an enormous impact on patient survival. Two approaches were developed by Marc Lippman (Washington). Firstly, hormone independent cells can overexpress *erb* B2 and the Georgetown group has identified the ligand that activates the membrane receptor. Novel *erb* B2 blocking drugs could become valuable new theraputic agents. Secondly, breast cancer cells secrete fibroblast growth factor-like substances that are necessary to allow tumour growth and homeostasis. Specific polysulphated molecules will prevent tumorigenesis in the laboratory and some have now entered clinical trial.

In a closing overview the successful transfection of the ER gene into hormone-independent breast cancer cells was described (V.C. Jordan, Madison). The growth of these stably transfected cell lines is inhibited by oestrogen; however pure anti-oestrogens have no effect on growth but block the inhibitory effect of oestrogen (S.Y. Jiang and V.C. Jordan, Madison). These novel studies may provide exciting therapeutic opportunities for the future. If a hormone refractory breast cancer cell can be "reinfected" with the ER gene through a targeted gene therapy then a weakly oestrogenic agent like tamoxifen could continue to control the growth of the disease. Indeed, the concept could be taken further. Perhaps any cancer could be infected with ER to provide a therapeutic advantage for the patient. It is possible that this is the dawn of a new era and the work of the molecular biologist can be focussed and targeted towards receptor pharmacology as a novel gene therapy.

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Multidrug Resistance from the Clinical Point of View

Coenraad K. van Kalken, Herbert M. Pinedo and Giuseppe Giaccone

INTRODUCTION

CHEMOTHERAPY is a curative treatment modality for several types of tumours even in advanced stages, like testicular cancer, a number of cancers of childhood and some haematological malignancies [1]. Other tumours such as ovarian cancer, acute myeloid leukaemia (AML), small cell lung cancer and advanced breast cancer, achieve high response rates and prolongation of survival by combination chemotherapy, but unfortunately they frequently relapse after an initial response and are then broadly resistant to drugs. Furthermore, common malignancies, like non-small cell lung cancer and colon cancer, are poorly responsive to chemotherapy from their diagnosis.

Causes of failure of chemotherapy are multifactorial and range from physical inability of the drugs to reach the critical cellular target (i.e. poor absorption, unfavourable pharmacokinetics and distribution, poor tumour vascularisation, low pH, etc.) to diverse cellular mechanisms of resistance. Drug sensitivity is intimately related to tumour growth kinetics and tumour volume; however, recently more emphasis has been given to the investigation of cellular mechanisms of resistance to drugs.

The hypothesis of spontaneous mutations as cause of drug resistance development, proposed by Goldie and Coldman in

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Table 1. *Characteristics of multidrug resistance types*

Type of MDR	Drugs involved	Mechanism	Gene/protein	Reversal
P-gp-mediated	Anthracyclines, vinca alkaloids, epipodophyllotoxins, colchicine, mitoxantrone	Decreased accumulation/changed intracellular distribution of drugs	*mdr*1/ P-glycoprotein	Calcium channel blockers, cyclosporin-A analogues, antimalarial drugs, calmodulin inhibitors, tamoxifen, others
Non-P-gp mediated	As above	As above	Unknown	Verapamil*
Topo II-mediated	Anthracyclines, epipodophyllotoxins, amsacrine, mitoxantrone, ellipticine	Decreased level/activity target enzyme	Topoisomerase-II	Unknown

*Verapamil shows very modest but significant effects on accumulation and cytotoxicity of MDR drugs in non-P-gp MDR cells. Clear reversal, however, has not been demonstrated for any compound thusfar [22, 23].

1979 [2], was corroborated by important scientific discoveries of the genetic changes in drug-resistant cells, as was suggested by studies on antibiotic resistance in bacteria [3]. The activation of several drug resistance genes through mutational events or amplification has been identified *in vitro* and in some clinical samples [1] and the recent discovery of the so-called multiple drug resistance (MDR) phenotype [4] is attracting broad interest because of its possible clinical implications. The *in vitro* selection of cell clones resistant to one of a group of natural product anticancer drugs (anthracyclines, vinca alkaloids, epipodophyllotoxins, colchicine) resulted in crossresistance to the whole group of drugs. Although it is still unclear whether the specific drug resistance pattern observed in *in vitro* selected resistant cells is representative of the clinical experience, where drug resistance seems to be a more general phenomenon, this model has proved excellent for testing drug resistance mechanisms in tumour cells.

Classical MDR cells (Table 1) showed a decreased accumulation of drugs [5] due to an increased ATP-dependent efflux [6]. P-glycoprotein (P-gp), a plasma membrane protein, was found to play a key role in the transport of drugs through the cell membrane [7] and so far two human *mdr* genes (*mdr*1 and *mdr*3) have been identified, encoding for two different P-gps [8]; however, although the genes are highly homologous, only *mdr*1 seems to confer multidrug resistance [9]. A characteristic of this type of resistance, which is being actively investigated, is the reversibility of resistance by drugs that modulate P-gp function. P-gp is highly conserved through species and is expressed in several normal human tissues: predominantly in organs possessing an excretory function (kidney, colon, liver), but also at several blood–tissue barrier sites, in the endometrium of the gravid uterus and in the adrenal. Although these findings fit the idea that P-gp plays a role in the protection of the organism against the effects of toxins, little is known about the actual physiological substrates of P-gp. A steroid transporting role has been suggested in the gravid uterus and the adrenal, where P-gp expression is high, but a decreased accumulation of steroid hormones in P-gp expressing cells of the endometrium has not yet been demonstrated [10]. Steroid hormones at least play a role in regulation of the gene expression [11].

P-GLYCOPROTEIN IN CLINICAL MULTIDRUG RESISTANCE

Haematological malignancies

Expression of *mdr*1-mRNA has been demonstrated in AML, acute lymphoid leukaemia (ALL) and in myelodysplastic syndromes. Overall the expression level was higher in chemotherapy treated leukaemia cases [12–15] and was correlated to refractoriness to treatment [14]. Recently *mdr*1 mRNA expression, detected by slot-blot technique, was demonstrated in 71% of 63 untreated AML patients; the complete remission rate to daunorubicin and cytarabine-containing chemotherapy was 89% in *mdr*1 mRNA negative and 53% in *mdr*1 mRNA positive cases [16]. Moreover, *mdr*1 mRNA expression was higher in M4 and M5 FAB leukaemia types and significantly correlated with a shorter disease-free and overall survival [16, 17]. Using a sensitive polymerase chain reaction (PCR)-based analysis, a comparable number of positive cases (27/51) among untreated AML patients were detected [18]. Musto *et al.* have shown that AML and ALL patients in complete remission, in whom P-gp positive cells were found in the bone marrow, relapsed rapidly with a high proportion of P-gp positive blasts [19]. Of interest, blasts of chronic myeloid leukaemia (CML) patients in blastic crisis showed a high expression of P-gp, whereas no or low expression was found during the chronic phase of CML [12, 14, 20]. In adult T-cell leukaemia, another entity highly refractory to combination chemotherapy, P-gp was expressed in the majority of patients (9/11) [21].

In cells of leukaemia patients with elevated *mdr*1 mRNA expression, a decreased accumulation of daunorubicin was observed, which could be restored by cyclosporin-A [15, 24]. Remarkably, daunorubicin accumulation could also be significantly enhanced by cyclosporin-A in cells from patients with prolymphocytic leukaemia, expressing *mdr*3 but not *mdr*1 [15, 25], although the human *mdr*3 gene does not seem to be involved in multidrug resistance [9].

Miller *et al.* have shown that P-gp expression was present in only 1/42 untreated patients with Hodgkin's or non-Hodgkin lymphomas [26]; however, 64% (7/11) of patients refractory to chemotherapy had an increased expression. The expression of P-gp in untreated myeloma patients was observed infrequently,

but a gradual increase of expression was seen after prolonged treatment [27–29] and this was correlated with the cumulative dose of doxorubicin plus vincristine administered.

Although the mentioned studies indicate a correlation between P-gp expression and refractoriness to chemotherapy in several haematological malignancies, the problem whether P-gp expression is an independent variable or a marker of a more malignant and consequently drug-resistant phenotype remains to be elucidated. Assays of the functional activity of P-gp, which can be readily performed on leukaemia and myeloma cells, will hopefully give more insight into this problem [30].

Solid tumours

Recent studies have demonstrated expression of the *mdr*1 gene in breast cancer [31–34]. A heterogeneous P-gp expression (1–10% of the cells) was detected in 28/29 primary breast cancer specimens by immunocytochemistry with monoclonal antibodies (Mab) C-219 and MRK-16 [31]. Interestingly, in addition to the staining of tumour cells, staining of stromal cells in the tumours was reported, detected in particular with the Mab C-219. The significance of this finding is as yet unclear and suggests caution in the interpretation of results of studies using bulk techniques. The expression of *mdr*1 at RNA [12] or protein level [33] in advanced breast cancer increased after exposure to chemotherapy, although no correlation was found with the type of treatment (with or without anthracyclines) [33]. The fact that non-MDR drugs [methotrexate, 5-fluorouracil (5-FU)] are usually part of combination chemotherapy regimens in breast cancer may indeed complicate the analysis of studies correlating expression of P-gp and response to chemotherapy. However, in fresh tumour cells from breast cancer patients a correlation was observed between P-gp expression and decreased *in vitro* cytotoxicity of doxorubicin and vincristine [28, 32, 33]. In a study of 20 patients with locally advanced disease, 17 patients expressed P-gp, assessed with the Mab C494 (specific for *mdr*1 P-gp) and intensive staining of >75% of cells appeared to correlate with a shorter progression-free survival [34]. In another study of 40 patients with locally advanced breast cancer, the expression of P-gp after primary chemotherapy significantly correlated with a poor response to chemotherapy [35].

High levels of *mdr* expression are mainly present in primary tumours originating from tissues which normally express the protein. Of interest, in renal cell cancer [36] and colorectal cancer [37] it was recently found that the level of expression correlated with the grade of differentiation of the tumour. Intriguingly, although renal cell cancer and Wilm's tumour originate from the same organ, the former is resistant to chemotherapy while the latter is sensitive. The expression of P-gp increased with the grade of differentiation of malignant tubules in both tumours, however the majority of cells of renal cell cancer stained positive, whereas the blastemal cells in Wilm's tumours failed to express the protein [36]. In addition, a significant increase in *in vitro* vinblastine and doxorubicin cytotoxicity with R-verapamil and trifluoperazine, related to the expression of P-gp, was found in cells obtained from patients with renal cell cancer [38, 39]. However, in a study of invasive cells at the leading edge of colorectal cancers P-gp expression was also found in 47/95 cases [40]. The authors reported a significantly greater incidence of vessel invasion and lymph node metastasis in P-gp positive cases, suggesting a correlation with local tumour aggressiveness and dissemination [40]. This study indicates that at least in colon cancer P-gp expression may not be simply related to differentiation alone.

An elevated P-gp expression, detected with immunocytochemistry, was highly predictive for early relapse and poor survival in children with embryonal sarcoma [41]. In another study in sarcomas, using a PCR-based technique, low to moderate expression of *mdr*1 mRNA was detected in the majority (70/86) of patients with osteosarcomas, chondrosarcomas or soft tissue sarcomas, tumours which are poorly responsive to chemotherapy [18]. Conversely, no expression was found in 6 Ewing's sarcomas, which are sensitive to chemotherapy [18].

Although *mdr*1 gene expression has been detected in both untreated and treated tumours, the clinical importance especially of very low levels of expression remains a matter of debate. Although with PCR it is now possible to amplify extremely low levels of *mdr*1 expression from tumour tissues [18], the less sensitive immunocytochemistry techniques allow the detection of heterogeneity and preferential localisation. The expression of P-gp in contaminating cells of the reticulo-endothelial system [40] and in stromal cells [31] may actually limit the usefulness of PCR and other molecular techniques. On the other hand, since crossreactivity has been described for antibodies reacting with P-gp, the use of a small panel of antibodies directed to different epitopes of the molecule is recommended.

CLINICAL TRIALS WITH RESISTANCE MODIFIERS

A significant problem of resistance modifiers is the toxicity encountered at the high doses presumably required to reverse resistance. Although a large number of modifiers have been tested *in vitro* (Table 1), still little is known of their *in vivo* activity. The recent development of transgenic mice expressing the *mdr*1 gene in the bone marrow might now provide the ideal model for rapid investigation of the bioactivity of potential reverters of multidrug resistance [43]. The bone marrow cells of these transgenic mice were resistant to MDR-drugs but not to methotrexate, 5-FU and cisplatin, and the *in vivo* reversing capacity of quinine, quinidine and verapamil was shown in this model [43].

Verapamil together with vincristine prolonged the life-span of mice bearing multidrug-resistant P388 xenografts and, when administered at high doses (plasma levels >10 μmol/l) accumulation of vincristine in P-gp-expressing organs such as liver, intestine and kidney was significantly increased. Significant modulating activity of R-verapamil on a nude mouse MDR colon carcinoma model was reported only at doses which were too toxic with the racemic mixture [44]. R-verapamil, the (+) isomer of verapamil is much less cardiotoxic than the L(−) isomer, although it seems to be equally effective in reversing resistance [45]. Increased activity of doxorubicin *in vivo* was also demonstrated with cyclosporin-A [44].

The results of early clinical trials with verapamil in solid tumours have been disappointing thusfar, one cause probably being the impossibility of adequate dose escalation due to prohibitive cardiovascular toxicity [46]. Nevertheless, 3 responses in 8 patients with myeloma or lymphoma resistant to VAD (vincrisinte, doxorubicin, dexamethasone) were reported with VAD plus verapamil (0.1 mg/kg bolus, 0.15–0.45 mg/kg/h during 132 h). 6 of these patients exhibited P-gp expression on their tumour cells [29]. 18 patients with non-Hodgkin or Hodgkin's lymphoma who had previously failed or relapsed within 3 months after a doxorubicin–vincristine-containing regimen were treated with CVAD (cyclophosphamide, vincristine, doxorubicin and dexamethasone) and verapamil (5-day continuous infusion at MTD). 13 (72%) responses, including 5 complete responses, were observed in this study [26]. However, clinical

resistance to the CVAD regimen and subsequent response with the addition of verapamil was clearly demonstrated in only 1 patient. Clinical studies in patients with relapsed leukaemia are ongoing and so far only one case report of a patient with AML in second relapse responding to reinduction therapy with daunorubicin and arabinoside–cytosine plus cyclosporin-A has been reported [47].

Other drugs have entered phase 1 or feasability studies [48–52]. In a study in 14 patients with advanced cancer using the calcium channel blocker bepridil (22 mg/kg in 36 h) in combination with an anthracycline, no bepridil-related cardiotoxicity was observed at plasma concentrations able to reverse MDR *in vitro* [4]. Although these patients were resistant to the same dose and schedule of the anthracyclines, 5 minor responses were observed, suggesting the potential of bepridil for further clinical evaluation. In a phase I trial with 72 h intraperitoneal etoposide in combination with dipyridamole in patients with ovarian cancer, 175 mg/m^2 per day of etoposide could be reached with a fixed dose of 24 mg/m^2 per day dipyridamole [49]. Dose-limiting toxicities were leukopenia and thrombocytopenia. In patients with breast cancer in whom epirubicin was combined with oral quinidine, cinchonism (dizziness, tinnitus, visual disturbances), nausea and lethargy related to quinidine were observed in 6/11 patient treated at higher dosages (500 and 1000 twice daily) [50]. In a phase I study with a combination of cyclosporin-A (15.6 mg/kg per day, given over 5 days) and vinblastine (1.2 mg/m^2 per day for 4 days) hyperbilirubinaemia, hypomagnesiaemia and cramps/constipation were observed [51]. Since the target organs for these side-effects express P-gp, the toxicity might have been due to interaction between P-gp and cyclosporin-A.

Tumours originating from P-gp expressing normal tissues (renal cell, colon and adrenocortical cancer) are intuitively regarded as good candidates for studies of reversal of resistance. Disappointingly, however, in the first clinical trial reported thusfar, no responses were reported in 15 patients with renal cell cancer with the combination of vinblastine (0.1 mg/kg) and cyclosporin-A (3 mg/kg as an 1 h infusion twice with a 6-h interval) [52]. Flushes were reported in 20% of the patients.

NON-P-GLYCOPROTEIN MEDIATED MULTIDRUG RESISTANCE

Other forms of resistance to multiple drugs have been described (Table 1). Cell lines displaying the multidrug-resistant phenotype without overexpression of *mdr*1-mRNA (non-P-gp MDR) have been selected *in vitro* [22, 23, 53–55]. During selection of SW 1573 lung cancer cell-lines for doxorubicin resistance, a non-P-gp MDR phenotype preceeded the expression of P-gp at lower concentration of doxorubicin [22, 23]. The selected clones showed resistance to the same group of anticancer drugs, although differences in crossresistance levels were found. As in classical MDR, several of the so-called non-P-gp cell lines show an accumulation defect, suggesting the existence of another active process extruding drugs from the cytoplasm. A characteristic pattern of membrane vesicle formation and release in resistant cells was observed in a non-P-gp mitoxantrone-resistant gastric carcinoma cell line [53]. Strong granular cytoplasmic staining was observed in these cells using a monoclonal antibody (LRP-56) which was raised against the non-P-gp MDR cell-line SW1573 2R120 [56]. The antibody also reacted with other non-P-gp cell lines [54, 55]. These studies suggest the presence of proteins which function as pumps at different localisations but with complementary mechanisms of action in the cell.

Topoisomerase II is the target of epipodophyllotoxins and several intercalating agents, like anthracyclines, m-AMSA, ellipticine and mitoxantrone [57, 58]. Reduction of the expression and the activity of the enzyme has been observed *in vitro* and *in vivo* to be correlated with a reduced cytoxicity to these drugs [58, 59]. The role of mutations of the topoisomerase II gene is still unclear [59, 60], although it would appear to play a minor role than regulation of expression levels of the enzyme.

Studies on the role of these mechanisms in clinical drug resistance have been initiated.

CONCLUSION

A number of studies have been published in recent literature on the detection of *mdr*1 expression in tumours and normal tissues. A correlation has been found between *mdr*1 expression and refractoriness to chemotherapy in several haematological malignancies and some solid tumours. Consequently clinical studies of several MDR reversing agents have been initiated in advanced cancer patients, and some of these have shown promising results, despite significant side-effects. However, there is still a considerable lack of information regarding the causative role of P-gp in chemotherapy refractoriness of patient tumour cells and, in particular the possible clinical significance of very low levels of expression of the *mdr*1 gene. A better knowledge of the functional activity of P-gp in tumours and the possibilities of its circumvention *in vivo* will hopefully lead to use of better reversing agents used at non-toxic concentrations. Finally, non-P-gp-mediated mechanisms of multidrug resistance have been described and further studies will be necessary to define their precise role in the everincreasing complexity of drug resistance.

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Community Lifestyle Characteristics and Lymphoid Malignancies in Young People in the UK

Freda E. Alexander, T. James Ricketts, Patricia A. McKinney and Raymond A. Cartwright

Data from a specialist registry of haematopoietic malignancies in England and Wales (1984–1988) have been analysed to investigate variations of incidence by age and diagnostic subtype of lymphoid malignancies in young people (aged 0–24 years). Attention has been focussed on the role of community lifestyle indicators for small areas, derived from routine sources, in an ecological analysis. The predominant conditions were acute lymphoblastic leukaemia (ALL)—42.4%, and Hodgkin's disease (HD)—37.5%. Lowest overall incidence at approximately 8 years of age corresponded to the termination of the childhood peak for ALL. Opposite trends of incidence rates with distance from urban centres (urban distance) were observed for the two age groups: odds ratios (OR) for areas >20 km from towns and cities were 1.46 (95% confidence interval 1.01–2.12) for ages 0–7 and 0.75 (95% confidence interval 0.56–0.99) for ages 8–24. For the younger group this was entirely attributable to ALL. HD, which was dominant in the older group, had highest incidence in connurbations but the gradient of risk for older onset ALL followed the overall pattern for this age group. A positive relationship with socioeconomic status was evident for both age groups but this was considerably stronger for the older cases (OR = 1.16, 95% confidence interval 1.01–1.33) than for the younger for whom it was not independent of urban distance. These results display an association between expression of lymphoid malignancies in young people and urban distance which is not attributable to socioeconomic status; for urban distances the distribution is shifted towards ALL and towards younger age at onset.
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INTRODUCTION AND BACKGROUND

THE AGE distribution of acute lymphoblastic leukaemia (ALL) and the overall incidence of disease have changed during the last half-century. The childhood incidence peak first observed in the UK [1] has emerged in other developed communities of diverse ethnic origin [2–4] and is believed to be associated with some aspects of social change [5]. Kinlen [6] found high rates in young children in areas where immigration was substantial and postulated an association with disregulated herd immunity. This would involve one or more specific agents but the Greaves hypothesis [7] suggested rather that general protection from antigenic challenge in infancy played a key role.

Similar cross-sectional [8] and secular [9] associations of the age-incidence pattern for Hodgkin's disease (HD) with socioeconomic factors have been documented. In conditions of poverty, childhood incidence was relatively high but in more affluent communities rates in children were generally lower, while a peak was found for young adults. It has been suggested [10] that this may have arisen as a result of delayed exposure to some relatively common infectious agent—the "late host response model". Considerable support for this was offered by both descriptive and analytical epidemiology [11, 12].

Although Greaves' hypothesis and the late host response model are entirely distinct, both suggest increased risk of disease for those protected from early infection. It was therefore appropriate to conduct parallel ecological analyses relating ALL

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