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Clinical Trials of P-glycoprotein Reversal in Solid Tumours

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INTRODUCTION

P-GLYCOPROTEIN IS a 1280 amino acid surface ATPase which can transport a wide range of cytotoxic drugs out of tumour cells, including, doxorubicin, vincristine, vinblastine, etoposide, actinomycin D, paclitaxel [1], vinorelbine [2], topotecan and irrinotecan [3]. The tumour cells which express P-glycoprotein have a decreased accumulation of substrate cytotoxics, and thus are resistant. However, an equally wide range of drugs, including verapamil, quinidine, cyclosporin A and tamoxifen, have been described as able to block transport of cytotoxic drugs out of cells [1, 4], and so can re-sensitise resistant cells to cytotoxics [5].

In cytotoxic cancer drug development, once active drugs have been identified in cell screens, xenograft studies follow [6]. One major problem with xenograft studies is that there is no guarantee that animal pharmacokinetics will be similar to those in humans. For trials of P-glycoprotein inhibitors, there is the additional problem that the pharmacokinetic interactions between inhibitor and cytotoxic need to be taken into account, and there is even less chance that animal pharmacokinetics will be predictive of those in humans.

P-glycoprotein expression is not the only mechanism in cancer cells which can confer drug resistance to chemotherapy [7]. However, because it is potentially surmountable by P-glycoprotein inhibitors, it remains a possible avenue for improving therapy. As more tumours are probed for P-glycoprotein expression, especially after chemotherapy [8], it is becoming clear just how widespread the clinical problem may be (Table 1). Other biological mechanisms of resistance to cytotoxics which are not surmountable by available technologies, such as mutation of *TP53* or *BCL-2* block of apoptosis, clearly do not currently offer any realistic prospect of improving patient treatment.

This review presents an analysis of reversal trials performed with P-glycoprotein inhibitors in solid tumours with the focus on the pharmacology of the P-glycoprotein inhibitors. The possibility of performing clinical trials utilising pharmacokinetic information, pharmacodynamic tests of P-glycoprotein inhibition and biopsy data of expression of P-glycoprotein are also discussed.

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IN VITRO ASSESSMENT OF P-GLYCOPROTEIN INHIBITOR POTENCY

There are numerous cell lines which express P-glycoprotein, and frequently these lines have been selected by exposure to cytotoxic drugs [9]. One reason such lines have been relatively easy to develop is the relatively high frequency with which P-glycoprotein-expressing lines can be generated, of the order 1×10^6 per cell generation [10]. Once such lines became available, it was possible to perform assays to quantify the potency of P-glycoprotein inhibitors. One assay simply measures the increase in the amount of intracellular cytotoxic drug substrate due to P-glycoprotein inhibitors, first reported for verapamil in P388 cells [11]. A modification of this approach is to utilise the fluorescent probe rhodamine, a method that can be automated to allow large scale screening [12].

The second cellular method is to perform proliferation assays (frequently MTT based) on P-glycoprotein-expressing cells in the presence and absence of P-glycoprotein inhibitors. This approach can even be semiquantified so EC_{50} values are derived [13].

The potency of P-glycoprotein inhibitors can be assessed in cell-free systems using inside-out vesicles (IOVs) from P-glycoprotein expressing cells [14]. By measuring the effects of inhibitors on the initial rate of ATP-dependent cytotoxic drug transport, clearly more potent drugs can be identified [14]. The most exact method of measuring potency of a drug to associate with a given target is to perform an equilibrium ligand binding assay in membrane particles. In the absence of transport against a concentration gradient, this assay allows K_i values to be accurately determined [15]. Table 2 lists all the drugs discussed in the following sections, giving their K_i values for the inhibition of [^{3}H]vinblastine binding to P-glycoprotein, and serum levels attainable. Ultimately, as for all drugs, *in vitro* potency and biophysical constants to bind to targets will relate to the potency of inhibitors in patients.

FIRST GENERATION P-GLYCOPROTEIN INHIBITORS

These clinical trials were performed with what was at hand at the time (see Figure 1 for structures). The rate of discovery of drugs which reversed MDR *in vitro* was rapid. Much was learned in these early ground-breaking trials; however, as can

Table 1. Detection of P-glycoprotein in solid tumours

Tumour type	Method of detection	Number positive/total or % positive	Reference
Adrenocortical	SBA	7/9	[102]
Bladder	HYB-241	Pretreatment 6/46 Post-treatment 6/11	[103]
Brain	C219 mRMA	0.3–15% cells 7/11	[104]
Breast	JSB-1 C219 C494	1/14 relapse patients before paclitaxel 7/14 relapse patients after paclitaxel	[8]
Cholangiocarcinoma	JSB-1	8/12	[105]
Colon	mRNA	35/41	[102]
Head and neck	UIC2	60%	[106]
Hepatocellular carcinoma	mRNA	12/12	[102]
Intermediate grade non-Hodgkin's lymphoma	C219	Untreated 1/19 Relapsed 11/23	[107]
Lung (NSCLC)	IHC	Smokers 42/72 Ceased smoking 2/22	[108]
Oesophagus	JSB-1	Squamous 1/27 Adenocarcinoma 7/10 Adenocarcinoma after chemotherapy 10/10	[109]
Ovarian	C219 JSB-1	5/20 untreated	[101]
Renal	HYB-241	5/6	[110]
Retinoblastoma	C494	29% untreated	[111]
Soft tissue sarcoma (adult)	MRK 16	12/22	[112]
Stomach	RT-PCR	4/12 (33%)	[113]
Testis	C219	SGCT 2/17 (12%) NSGCT 20/38 (53%)	[114]
Thyroid (anaplastic)	mRNA	20/20 (low level)	[115]

SBA, slot blot analysis; SGCT, seminomatous germ cell tumour; NSGCT, non-seminomatous germ cell tumour; NSCLC, non-small cell lung cancer; HYB-241, C219, JSB-1, C494, MRK16 and UIC2 are monoclonal antibodies.

Table 2. P-glycoprotein inhibitors

Inhibitor	Dose and route	Dose-limiting toxicity of inhibitor	Serum levels in phase I trial [Ref.]	K_i to bind to P-glycoprotein (nM)
Amiodarone	1400 mg/day for 7 days	Negative dromotropic	8.5 μM [116]	2000*
Bepredil	22 mg/kg/36 h CIV	Not defined	5.3 μM [117]	800*
CGP 41251	Oral	Not known	Trial in progress	42 [94]
Cyclosporin A	2–7 mg/kg IV over 2 h then 5–21 kg/kg/day, 3 days	Elevated bilirubin	0.28–5 μM [26]	17 [15]
Dexniguldipine	5 mg/kg over 4 h IV	Hypotension	0.5 μM [74]	37 [118]
Dexverapamil	225 mg/m^2 every 4 h orally	Hypotension bradycardia	Peak 6.2 μM [65]	600 [15]
GF 120918	Orally	Not known	Trial in progress	3†
Nifedipine	60 mg orally twice daily	Headache, postural hypotension	0.6 μM [119]	5000 [15]
Progesterone	4 g IV over 24 h	Deep sleep	5 μM [96]	200 000*
PSC 833	2.5–5 mg/kg up to 4 days	Elevated bilirubin	0.8 μM [90]	35*
S9788	40 mg/m^2 IV over 30 min	MTD not defined	1.2 μM [80]	250*
Quinidine	250 mg twice daily orally, 4 days	QRS interval prolongation	6 μM [39]	274 [15]
Quinine	24–30 mg/kg/day orally	Tinnitus, vertigo	30 μM [43]	1912 [15]
Tamoxifen	480 or 720 mg/day for 6 days	Cerebellar	4 μM [50]	800*
Verapamil	0.15–0.60 mg/kg/h, 4 days	Hypotension, heart block, fluid retention	0.9–2.3 μM [17]	600 [15]

*Ferry and Russell, unpublished observation; †Ferry and coworkers to be presented at the American Association for Cancer Research, 1996. CIV, continuous intravenous infusion.

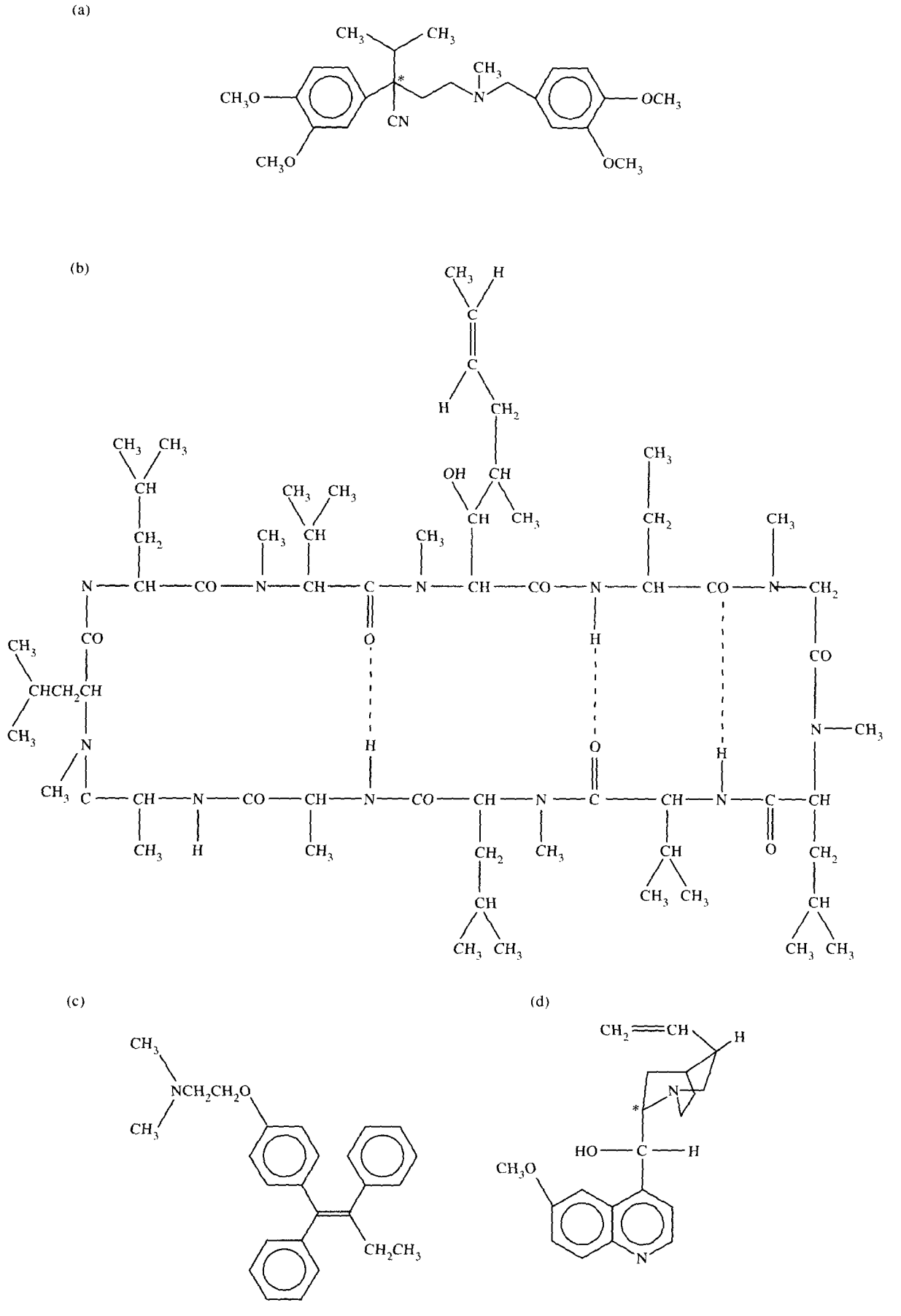


Figure 1. First generation P-glycoprotein inhibitors. (a) Racemic *verapamil*; (b) *cyclosporin A*; (c) tamoxifen; (d) quinidine and *quinine*. The asterisks designate chiral carbon atoms.

be seen in Table 2, many of these first generation drugs have only μM affinity to bind to P-glycoprotein.

Verapamil

Verapamil is a phenylalkylamine, first developed as a use-dependent blocker of voltage-dependent L-type calcium channels, which proved efficacious in angina, hypertension and supraventricular tachyarrythmia. Verapamil has one chiral centre and is a mixture of R-verapamil ((+)-, D-verapamil or dexverapamil) and S-verapamil ((−) or -verapamil). It was one of the first agents described which inhibits P-glycoprotein-mediated resistance to vincristine and vinblastine [11]. Not surprisingly, because of its clinical availability, trials with verapamil were initiated.

Early clinical trials indicated that the maximum tolerated dose (MTD) dose of verapamil was 480 mg/day orally, which lead to blood levels of 1 μM [16]. Dose limiting toxicity (DLT) was hypotension [16]. Dose escalation studies with intravenous verapamil have also been peformed in patients receiving concomitant 4 day infusions of doxorubicin, vincristine and dexamethasone (VAD) [17]. These confirmed that, at the dose range 0.15–0.6 mg/kg/h, cardiovascular toxicities were dose limiting, but, for example, heart block could easily be controlled by temporarily discontinuing the infusion [17]. Oedema and weight gain were also seen. The median verapamil level achieved in 35 patients who received 71 courses was 397 ng/ml (0.9 μM) [17].

In a subsequent phase II clinical trial of intravenous verapamil with VAD plus cyclophosphamide (CVAD) in non-Hodgkin's lymphoma resistant to chemotherapy, the range of verapamil concentrations achieved in 18 patients ranged between 0.68 and 4 μM, with a median of 2.3 μM [18]. Because of the design of the CVAD trials, it was not possible to evaluate the effects of verapamil on the pharmacokinetics of the co-administered doxorubicin and vincristine.

Little is known about the pharmacokinetic interactions of verapamil with cytotoxic drugs which are P-glycoprotein substrates. Oral verapamil has been reported to increase the peak levels, prolong the terminal half-life and increase volume of distribution at steady state of doxorubicin [19]. Different conclusions were reached with epirubicin in a clinical trial in which two groups of patients were treated with (i) epirubicin 40 mg/m^2 for 3 days (ii) epirubicin 40 mg/m^2 for 3 days with verapamil 480 mg per day for 4 days [20]. These authors found that verapamil increased the AUC of seven deoxyaglycone metabolites of epirubicin.

Because verapamil undergoes extensive hepatic metabolism, it is subject to a marked first pass effect of 85% after oral dosing [21]. Therefore, on theoretical grounds, if verapamil is infused directly into the hepatic artery, high hepatic exposure should occur. In a clinical trial of 14 patients, verapamil was given as a 14 h hepatic artery infusion and the MTD was 1.2 mg/kg/h with hypotension being the dose limiting toxicity (DLT) [22]. The estimated concentration of verapamil delivered to hepatic tumours was 7.3 μM, approximately 3-fold higher than with maximally tolerated intravenous administration.

To our knowledge, there has been only one randomised trial of verapamil added to chemotherapy, in small cell lung cancer [23]. The trial accrued 226 patients who were treated with cyclophosphamide, doxorubicin, vincristine and etoposide (CAVE) [23]. The dose of verapamil was 480 mg/day orally for 5 days. The serum levels of verapamil were 0.8 μM, which is 7.5-fold lower than needed to reverse P-glycoprotein mediated resistance *in vitro* [23]. The other problem with this clinical trial is its small size which could only reliably detect a difference in median survival of approximately 30%.

The pharmacokinetic complexities of combining chemotherapy with verapamil have been appreciated for some time, yet there are still no data concerning the pharmacokinetic interaction of verapamil with vinca alkaloids, paclitaxel or etoposide. The data we have with anthracyclines is contradictory. Because P-glycoprotein inhibitors which are two orders of magnitude more powerful than verapamil (*vide infra*) are now available for clinical trials, it is, therefore, unlikely that these questions will ever be systematically addressed.

Cyclosporin A

Cyclosporin A is a cyclic unadecapeptide which has immunosuppressive activity, and its modulation of P-glycoprotein-mediated drug resistance has been reviewed [24]. Cyclosporin A inhibits the drug metabolising enzyme cytochrome P450, especially the CYP3 isoform [25], suggesting it may cause significant pharmacokinetic interactions. Detailed studies combining cyclosporin A and etoposide [25], doxorubicin [26] and paclitaxel [27] have been published documenting such interactions.

There is very little animal data on the modulation by cyclosporin A of doxorubicin pharmacokinetics, but that which is published should encourage clinical investigators. The studies have been conducted in male mice and rats given a single i.p. injection of cyclosporin A and 30 min later given i.v. doxorubicin at 5 mg/kg [28]. Although serum levels of doxorubicin were not altered by cyclosporin A, the AUC of liver, kidney and adrenals increased 2–3-fold [28]. Thus, even in the absence of significant increases in serum levels of a cytotoxic drug, tissue and hopefully tumour content could be significantly altered. This suggests that, in clinical trials of solid tumours, a non-invasive method of measuring tumour levels of cytotoxic drugs would be valuable to determine tumour drug AUCs, as well as serum level measurements.

In the etoposide/cyclosporin A trial, patients first received IV etoposide at doses of 150–200 mg/m^2 for 3 days and, if they progressed, were then switched to the etoposide/cyclosporin A combination. Cyclosporin A was given as a loading dose of between 2 and 7 mg/kg as a 2 h infusion, followed by a 3 day infusion of 5–21 mg/kg/day. Because of the variable cyclosporin A doses, a range of steady state plasma levels between 297 and 5073 ng/ml (0.28–5 μM) were achieved. In patients with higher cyclosporin A levels, greater increases in etoposide AUC were seen, with a median of 50% for patients with levels < 2 μM and 80% for those > 2 μM [25]. Etoposide has cycle-phase specific antiproliferative effects and it is therefore important to note that when cyclosporin A levels were > 2 μM the $t_{1/2}$ increased by 108%.

The first report of cyclosporin A modulation of doxorubicin pharmacokinetics used a 10 min bolus injection of doxorubicin, and reported a 10-fold decrease in doxorubicin clearance [29]. Because the design did not assess paired pharmacokinetic data versus doxorubicin given alone, this trial left considerable doubt on the magnitude of the effects of cyclosporin A. In a subsequent phase I clinical trial, the effects of cyclosporin A were evaluated in a sequential design [30]. Cyclosporin A was given i.v. as a loading dose of 6 mg/kg over 2 h, then as an infusion at 18 mg/kg/day for 3 days. The dose of doxorubicin alone was at 60–75 mg/m^2 given as a 48 h infusion. When

doxorubicin was combined with cyclosporin A, the starting dose was reduced to 40%, escalating to 70%. Equivalent myelosuppression for the combination was seen at 60% doxorubicin doses, which correlated well with the increase in doxorubicin AUC of 55%. Both renal and non-renal clearance decreased accounting for the increased AUC. The AUC of the metabolite doxorubicinol increased by 3.5-fold, probably in part because doxorubicinol is metabolised by cytochrome P450 demethylation [31], and cyclosporin A is known to inhibit these enzymes [32].

Cyclosporin A has also been combined with VAD in myeloma [33]. This trial treated 21 refractory myeloma patients with three doses of cyclosporin A, 5, 7.5 and 10 mg/kg/day. In 6/8 patients in whom *MDR1* was detected prior to therapy the *MDR1* expressing clone became undetectable and overall 48% of the treated patients responded. No systematic pharmacokinetic data for doxorubicin or vincristine were collected, but the AUC ratio of doxorubicinol to doxorubicin was increased as reported for infusional doxorubicin studies [26].

Attempts have been made to combine vinblastine given 0.1 mg/kg weekly with cyclosporin A given twice daily as a 1 h infusion at 3 mg/kg [34]. The median 18 h blood level of cyclosporin A was 0.6 μM and no vinblastine kinetics were measured. The trial enrolled 15 patients and no responses were seen. The reasons for treatment failure are unknown. Certainly the schedule and levels of cyclosporin A were suboptimal compared with more recent reports, but the absence of vinblastine kinetics makes it impossible to come to any firm conclusions.

There is clearly a need to define the pharmacokinetic interactions of cyclosporin A with cytotoxic drugs more accurately if hypothesis-testing trials comparing equi-AUC doses of P-glycoprotein substrates (or combinations) are to be performed. In this context, it is worth considering the potential complexities of combining P-glycoprotein inhibiting drugs, as has been suggested on the basis of laboratory work [35] and actually undertaken with tamoxifen [36]. Not only may both the P-glycoprotein inhibitors modulate cytotoxic drug kinetics, but may also alter each others disposition making evaluation difficult.

Quinidine

Quinidine is a naturally occurring alkaloid used since the 1950s as an anti-arrhythmic drug. It was one of the first drugs to be shown to modulate P-glycoprotein transport of cytotoxic drugs [37]. In a unique study, patients received quinidine prior to undergoing breast cancer surgery [38]. Tumour tissue levels of quinidine were comparable with those in plasma, i.e. around 6 μM [39].

This work led to a trial of 223 patients who were randomised to receive epirubicin at 100 mg/m^2 and prednisolone as a bolus dose, or epirubicin/prednisolone plus quinidine orally 250 mg twice daily for 4 days [38]. One biological factor to consider is the recognised effect of corticosteroids in increasing expression of P-glycoprotein *in vitro* [40]. If these effects are operative *in vivo*, the use of prednisolone may have increased the expression of P-glycoprotein, blunting any activity of quinidine.

The trial did not assess epirubicin pharmacokinetics, but quinidine levels were 5.5 μM directly before epirubicin administration. No differences in survival or toxicity were seen. Furthermore, no tumour biopsies were assessed for P-glycoprotein expression, but other work suggests that only 10–20% of breast cancers express P-glycoprotein at diagnosis [41]. This means that, in each arm of the quinidine/epirubicin versus epirubicin trial of 223 patients, only approximately 22 patients would be P-glycoprotein positive. This randomised trial was small and only able to detect reliably an increase in response rate from approximately 40 to 60%. The fact that the majority of patients are likely to have been P-glycoprotein negative means its true power to detect a difference may well have been substantially less than the initial power calculations predicted. Quinidine is a relatively weak inhibitor of P-glycoprotein transport (Table 2) and it would seem to be an unlikely candidate for further development.

Quinine

Quinine is the *S*-diastereoisomer of quinidine. In functional assays of P-glycoprotein transport of rhodamine, it is weaker than quinidine [42], and has a 7-fold lower affinity for inhibiting equilibrium binding of [^{3}H]vinblastine [15]. Quinine is used clinically to relieve nocturnal leg cramps and treat malaria.

In an *ex vivo* assay of quinine activity, serum was taken from patients who received 24–30 mg/kg/day orally, and serum levels of quinine ranged from 4.8 to 10 μg/ml (15–31 μM). This serum was able to increase the epidoxorubicin content of DHD/K12 rat colon cells, suggesting that the serum levels achieved were sufficient to inhibit P-glycoprotein function. This *ex vivo* activity has been confirmed with patient serum in resistant K562/ADM cells [43]. The reason why quinine is weak *in vitro* is because most of the activity from serum is attributable to the metabolite quinine-10,11-epoxide, which is 8-fold more active than quinine in a rhodamine efflux assay [42].

Quinine has been combined with the P-glycoprotein substrate mitoxantrone and arabinose C in acute leukaemia [44]. Quinine at 30 mg/kg was given by continuous i.v. infusion for 5 days. The quinine related toxicity was tinnitus and vertigo in 10 of 15 patients. Of 3 patients assessed, 5 cases were P-glycoprotein positive and they all responded. Surprisingly, little is known about the pharmacokinetic interactions of quinine and cytotoxic drugs. There may be a possible decrease in epirubicin AUC [45].

Some data with quinine are encouraging, but it seems likely that the epoxide metabolite is responsible for any biological effects. This adds an unwanted level of complexity. How variable is the metabolism of quinine? Why not use the more active metabolite? Furthermore, previously it has been suggested that verapamil and quinine acted synergistically to reverse P-glycoprotein mediated resistance *in vitro* [46]. Apart from the added complications dual inhibitor studies impart to interactions with cytotoxics, there is the question of whether the quinine/verapamil synergy applies to the quinine-epoxide/verapamil combination, especially as the quinidine/verapamil combination was antagonistic [46].

Tamoxifen

Tamoxifen is an oestrogen receptor antagonist, first reported in 1991 to be active in breast cancer at doses of 20 mg per day [47]. The pharmacokinetics of tamoxifen are complex, with a terminal half-life of excretion of 5–7 days, primarily due to very tight plasma binding (approximately 98%), an enterohepatic circulation and accumulation in fatty tissue as expected from a highly hydrophobic drug.

Tamoxifen binds weakly to P-glycoprotein, with K_i value of

0.8 μM (Table 2), and binding to P-glycoprotein increases ATPase activity [48]. Because modulation of P-glycoprotein-mediated resistance in cell lines is effected only at > 1 μM [49], clinical trials have focused on increasing the dose of tamoxifen above that used for endocrine therapy of breast cancer.

In a phase I dose-escalation trial in combination with vinblastine, the MTD of tamoxifen was a loading dose of 680 mg/m^2 followed by 260 mg/m^2 twice daily for 13 days [50]. The dose limiting toxicities were tremor, unsteady gait, dizziness, and one patient had a grand mal seizure. At the MTD, plasma tamoxifen (and major metabolite *N*-desmethyltamoxifen) levels were in the range 4–8 μM. In this trial, two tumour biopsies were performed and tissue levels were approximately 6-fold higher than in plasma. This trial accrued 53 patients, 1 partial response was seen in renal cell cancer, and two in breast cancer. No pharmacokinetic analysis of the effects of tamoxifen on vinblastine were presented, but the nadir white cell and platelet counts were no worse between the lowest dose of tamoxifen and the 6.5-fold highest dose [50].

A clinical trial of similar design combined 'high-dose' tamoxifen, 480 or 720 mg per day for 6 days, with oral etoposide [49]. The CNS toxicities were similar to those reported when tamoxifen is combined with vinblastine [50]. The tamoxifen levels achieved, approximately 4 μM, were ineffective in modulating etoposide resistance in P-glycoprotein expressing cell lines [49].

In a randomised phase II trial in renal cell cancer, patients received infusional vinblastine for two courses, and if they progressed or had stable disease, were randomised to receive either vinblastine plus tamoxifen (400 mg loading, then 150 mg twice daily for 13 days) or cyclosporin A 12.5 mg/kg/day for 5 days. In 64 patients no responses were seen with vinblastine alone. With addition of tamoxifen, 7 of 16 progressing patients stabilised compared with 4 of 12 given cyclosporin A [51]. This trial with sequential design has the possibility of isolating the effect of the added modulator, provided alterations in vinblastine pharmacokinetics do not confound interpretation.

Tamoxifen, at 150 mg/m^2 twice daily, has been given to patients receiving combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, prednisolone and etoposide (CHOPE) [52]. The authors suspected worsening myelosuppression due to addition of tamoxifen, but because the trial was not of sequential design, the reasons for this could not be rigorously assessed.

Laboratory findings of synergy between tamoxifen and cyclosporin A in reversing MDR have been reported [36]. At the highest dose of tamoxifen, 300 mg/m^2 twice daily, cerebellar ataxia was observed. The main toxicity of cyclosporin A was mild hyperbilirubinaemia, which correlated with serum cyclosporin A levels [36].

Thus far, no clinical trials have assessed the effect of tamoxifen on pharmacokinetics of cytotoxic drugs. Furthermore, even if activity is seen, tamoxifen is also known to inhibit protein kinase C (PKC) at high μM concentrations [53]. P-glycoprotein is known to have PKC phosphorylation sites in the linker region, but key experiments comparing transport of wild type with the non-phosphorylatable mutant have not yet been reported. However, it should also be borne in mind that tamoxifen could circumvent P-glycoprotein-mediated resistance by decreasing *MDR1* mRNA, as has been reported for other kinase inhibitors [54]. In addition, phosphatidylethanolamine may be involved in transport of drugs by P-glycoprotein, and the inhibitory effects of tamoxifen on synthesis of this phospholipid may be important [55].

Because of its protein biological activities at high doses tamoxifen is not a promising candidate to test the hypothesis that P-glycoprotein mediates clinical drug resistance. Furthermore, if rational trials are to be performed with tamoxifen in the future, then fundamental pharmacokinetic work needs to be undertaken first.

SECOND GENERATION P-GLYCOPROTEIN INHIBITORS

These drugs were thought likely to have less toxicity than the first generation of inhibitors. Their structures are shown in Figure 2.

Dexverapamil (R-verapamil)

Dexverapamil is 7-fold less potent than l-verapamil at inhibiting the contractile force of isolated human myocardium, with an EC_{50} of 3 μM [56]. However, the enantiomers of verapamil are equipotent at reversing uptake-deficit of cytotoxic drug [57, 58] and reversing P-glycoprotein-mediated resistance [59].

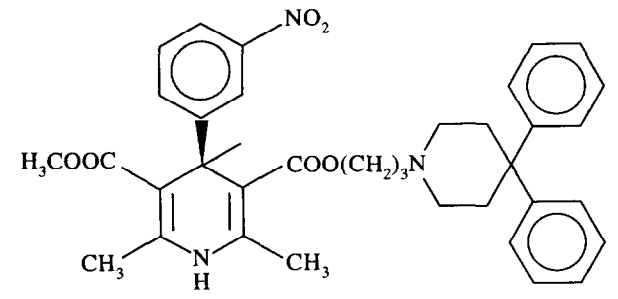
In a phase I clinical trial, dexverapamil was administered orally with doxorubicin given as a bolus every 3 weeks at 70 mg/m^2 [60]. Dexverapamil was given in divided doses four times daily, but at a total daily dose of 1200 mg, 5 of 6 patients had symptomatic hypotension with systolic blood pressures of < 100 mmHg. This trial recommended a daily dose of 800 mg daily for phase II trials, but the plasma levels of 1.9 μM plus those of the equiactive metabolite norverapamil of 1.8 μM were probably too low to inhibit P-glycoprotein function.

In a more recent phase I clinical trial, oral dexverapamil was combined with infusional etoposide, doxorubicin, vincristine, oral prednisolone and bolus cyclophosphamide (EPOCH) [61]. This trial recommended somewhat higher doses of dexverapamil of 900 mg/m^2/day for 6 days, at which 3 of 26 patients had dose reductions because of cardiovascular toxicity [61]. The peak levels of dexverapamil achieved was 1.7 μM, and the sum of dexverapamil and norverapamil concentrations was 3.24 μM. In this trial, the important issue of effect on cytotoxic drug pharmacokinetics was assessed by comparing steady state concentrations of etoposide and doxorubicin for EPOCH with dexverapamil plus EPOCH. There was no effect on etoposide C_{SS}, but that of doxorubicin doubled [61]. Vincristine pharmacokinetics could not be evaluated because the levels would be at the limit of assay sensitivity. No elevation of serum bilirubin was noted, but adding dexverapamil to EPOCH decreased platelet and neutrophil nadir counts. Despite the low plasma levels of dexverapamil achieved in a phase II trial in refractory lymphoma, 5 of 41 patients progressing on EPOCH responded when dexverapamil was added, and this correlated with *MDR1* mRNA tumour biopsy expression [62].

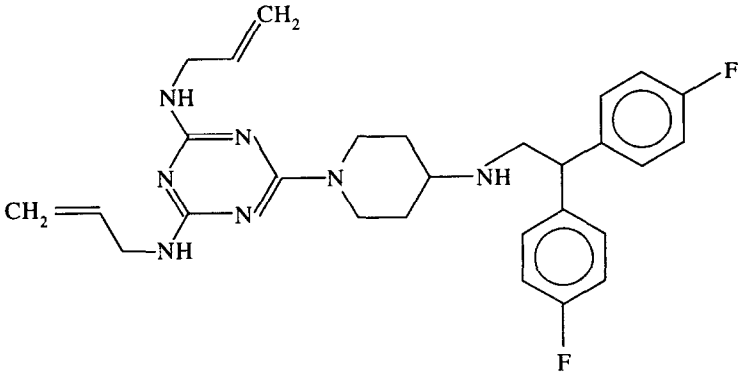
Dexverapamil has been combined with vinblastine in clinical trials in renal cell carcinoma. In one report, oral doses of 180 mg/m^2 four times daily led to hypotension in only 1 of 11 patients [63], but in another trial dexverapamil doses as high as 3000 mg/day were tolerated by 8 of 11 patients, with 1 patient responding to the addition of dexverapamil [64]. The effects of dexverapamil on vinblastine pharmacokinetics are unknown.

Dexverapamil has also been given with paclitaxel in breast

(a) Dexniguldipine



(b) S9788



(c) Dexverapamil

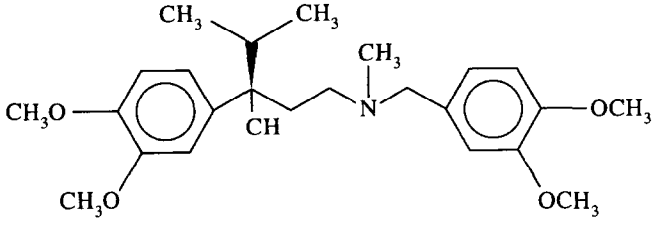


Figure 2. Second generation P-glycoprotein inhibitors.

cancer patients [65]. Dose-limiting bradycardia and hypotension were seen at a dexverapamil dose of 250 mg/m^2 every 4 h; but 225 mg/m^2 every 4 h with paclitaxel at 200 mg/m^2 was well tolerated. Peak and trough levels of verapamil were high, 6.2 and 3.6 μM. Dexverapamil worsened paclitaxel myelosuppression. Of 7 patients, 3 had minor responses when dexverapamil was added to paclitaxel. However, because dexverapamil increased the paclitaxel AUC 2-fold, by decreasing clearance [65], it is possible the increased tissue exposure to paclitaxel caused the observed antitumour effects.

The doses of dexverapamil which have caused cardiac toxicity have not been as high as previously anticipated. This may be because dexverapamil undergoes selective metabolism relative to l-verapamil, and the bioavailability of dexverapamil is twice that of l-verapamil [66]. Therefore, as 25% of the cardiodepressant effect of racemic verapamil is due to dexverapamil [56], it is not surprising that the dose escalation achieved by using this enantiomer has mostly been modest.

Dexniguldipine

Dexniguldipine (B8509-035) is the (−)-isomer of the antihypertensive agent (+)-niguldipine [67]. Dexniguldipine binds with high affinity to P-glycoprotein with a K_i of approximately 10 nM [68]. The binding site for dexniguldipine on P-glycoprotein is located on an intracellular domain [69]. Niguldipine is also a blocker of L-type calcium channels, alpha adrenergic receptors [67] and protein kinase C at > 3 μM, by interacting with the regulatory domain [70]. Dexniguldipine has been reported to have antitumour effects in neuroendocrine tumour xenografts, and to inhibit RNA synthesis at 5 μM [71]. In functional assays of P-glycoprotein transport of rhodamine, 1 μM dexniguldipine is maximally effective [72].

Because of the xenograft data with deniguldipine, a phase I dose escalation trial was undertaken in cancer patients. Up to doses of 2500 mg/day, only grade 2 nausea was reported, only 2 patients had hypotension, no responses were seen, and 1 patient, with a tumour of the maxillary sinus, remained on therapy for 23 months with stable disease [73].

In phase I trials, intravenous dexniguldipine has been combined with vinblastine [74]. Dexniguldipine was given as a 4 h infusion on 4 consecutive days, and the dose-limiting toxicities were hypotension and cardiac conductance disturbances at the end of the infusions, the MTD was 5 mg/kg/day. Peak plasma levels were 2 μM, with steady state levels around

0.5 μM. In combination with vinblastine, one partial response was seen in a patient with retroperitoneal mesothelioma.

Although dexniguldipine is 40-fold less potent than niguldipine at blocking L-type calcium channels, cardiovascular toxicity prevented dose escalation to levels which would be required to reverse MDR. Dexniguldipine is a highly hydrophobic drug which undergoes hepatic metabolism. It may therefore still find a niche use in regional chemotherapy of P-glycoprotein-expressing liver tumours.

S9788

This triazionoaminopiperdine derivative was chosen from a programme to develop potent P-glycoprotein inhibitors [75]. S9788 is approximately 5-fold more potent than verapamil at increasing accumulation of doxorubicin in P-glycoprotein-expressing cell lines [76]. S9788 blocks photoaffinity labelling of P-glycoprotein by [^{3}H]azidopine at 100 μM. In a comparative study, S9788 caused complete sensitisation of P-glycoprotein-expressing cell lines at 10 μM, values for other reversing agents being: cyclosporin A 3 μM, verapamil 6 μM, amiodarone 20 μM, tamoxifen 40 μM and quinidine > 100 μM [77]. Detailed work using laser confocal microscopy has demonstrated that S9788 can cause THP-doxorubicin to accumulate in the nuclei of resistant K562R cells, and after brief exposure to 5 μM S9788, increased the half-life of THP-doxorubicin in the nucleus to 90 min, versus 20 min for 5 μM cyclosporin A [78].

S9788 has been found to have activity in reversing resistance P-glycoprotein-expressing small cell lung cancer xenografts treated with etoposide/cisplatin at a dose of 50 mg/kg twice per day for 3 days with etoposide [79].

In a phase I clinical trial, S9788 was combined with doxorubicin in a sequential design to allow treatment with doxorubicin alone, S9788 (IV over 30 min) alone and then both drugs combined. The starting dose of S9788 was 8 mg/m^2 and was reported to be escalated to 96 mg/m^2 [80]. No serious toxicities were found and the combined treatment did not seem to worsen haematological toxicity of doxorubicin. Very little pharmacokinetic data were reported, but at 40 mg/m^2 the peak serum level of S9788 was 1.2 μM [80]. Further clinical trial data are awaited.

THIRD GENERATION P-GLYCOPROTEIN INHIBITORS

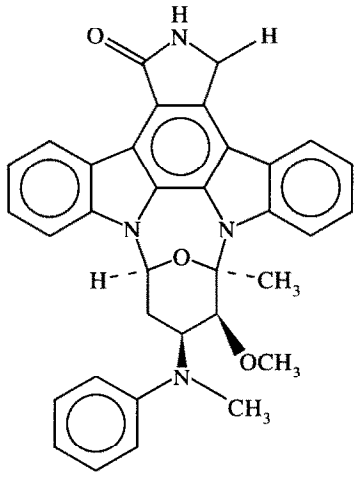
This class of inhibitor is characterised by low nM potency for reversing P-glycoprotein activity *in vitro* (Figure 3), these compounds and those which follow in the future are likely to be the product of specific drug discovery programmes.

PSC 833

PSC 833 is a non-immunosuppressive analogue of cyclosporin D. In comparative *in vitro* studies in P-glycoprotein-expressing cell lines, it is reported to be equipotent [81] to 20-fold more potent than cyclosporin A [82, 83]. *In vitro*, PSC 833 competes for [^{3}H]-vinblastine binding to P-glycoprotein with a K_i value of 35 nM (Table 2) and also potently inhibits P-glycoprotein photoaffinity labelling. PSC 833 has a K_i value of 300 nM for inhibiting transport of daunomycin in *in vitro* transport assays, but also inhibits the taurocholate transporter with a K_i of 600 nM and the leukotriene transporter (encoded by the *MRP* gene) with a K_i of 30 μM [84].

In xenograft studies of the L1210 P-glycoprotein-transfected cells, PSC 833 was effective together with doxorubicin

(a) CGP41251



(a) GG918

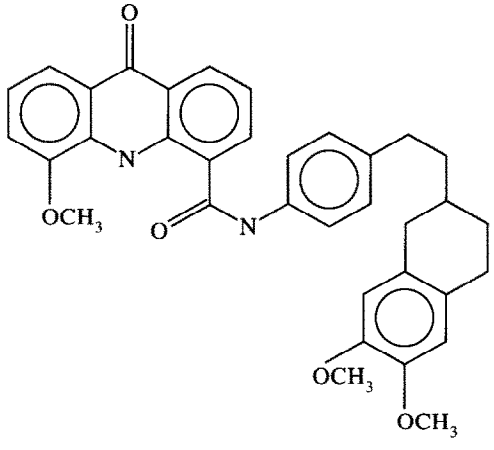


Figure 3. Third generation P-glycoprotein inhibitors.

at prolonging survival and some cures were observed [85]. However, PSC 833 strongly interacted with the pharmacokinetics of doxorubicin, lowering the MTD of doxorubicin 3-fold [85].

Phase I clinical trials have been undertaken with PSC 833 and cytotoxic drugs, including single agent etoposide [86], doxorubicin [87, 88] and paclitaxel as well as with the VAD combination [89]. As for cyclosporin A, the AUCs of etoposide, doxorubicin and paclitaxel are increased, increasing myelosuppression. To allow increases in PSC 833 doses above 5 mg/kg twice daily, doxorubicin doses have been decreased [87, 88]. Despite the early nature of the clinical trials underway, bioassays on serum from PSC 833 treated patients have shown samples have sufficient PSC 833 (in the range 0.4–0.8 μM) to reverse partially the resistance of the MDR cell line MAES-SA/Dx5 [90].

As for cyclosporin A, transient hyperbilirubinaemia has been seen when PSC 833 is combined with cytotoxic chemotherapy, possibly due to inhibition of transporters other than P-glycoprotein in the bile canalicular membrane [84]. Clinical trial results with PSC 833 are awaited with considerable interest.

CGP 41251

Human P-glycoprotein has potential PKC phosphorylation sites at serine 661, 671, 667, 675 and 683 [91]. CGP 41251 is the N-benzyl derivative of staurosporine (Figure 3). It has a degree of selectivity for PKC and in xenografts antitumour activity [92]. In P-glycoprotein expressing CCRF-VCR1000 cells, CGP 41251 has antiproliferative activity (IC_{50} 420 nM) and strongly potentiates the effects of doxorubicin and vinblastine [93]. In the same cells, the IC_{50} for increasing accumulation of rhodamine is 50 nM, close to the K_i value of CGP 41251 for inhibiting binding of [^{3}H]vinblastine to P-glycoprotein [94]. Unlike the effect of staurosporine, CGP 41251 does not decrease expression of P-glycoprotein [93].

Thus, CGP 41251 seems to bind with high affinity to P-glycoprotein to block drug transport [94], but the role of PKC inhibition is unclear, although it is likely to involve antiproliferative effects.

CGP 41251 is currently in phase I clinical trial as an MDR reversing agent in the United Kingdom. These clinical trials will be extremely challenging, as CGP 41251 is unique amongst potent P-glycoprotein inhibitors in having potent antitumour activity. This may mean it is not the ideal P-glycoprotein inhibitor to test if this particular mode of resistance is clinically significant.

GF 120918 (*GG*918)

This acridonecarboxamide was developed by Glaxo (France) and is the most potent P-glycoprotein inhibitor described, having an EC_{50} for reversing P-glycoprotein-mediated drug resistance of 20 nM *in vitro* [95]. Furthermore, in xenograft experiments, 5 mg/kg GG918 restores sensitivity of P388/Dox tumours to doxorubicin without altering pharmacokinetics of doxorubicin [95]. Results of ongoing phase I clinical trials in North America and Europe should become available in 1997.

CONCLUSION

It is curious that the dose limiting toxicities (DLTs) of clinically tested P-glycoprotein inhibitors have varied (Table 2). For first generation drugs, DLTs were related to primary pharmacological activities, cardiovascular for verapamil, tinnitus for quinine. However, tamoxifen and progesterone caused unusual DLTs, cerebellar ataxia [50] and solmonelence [96], respectively. Cyclosporin A and its analogue PSC 833 both cause elevation of serum bilirubin, but is not clear if this effect is due to P-glycoprotein inhibition, as cyclosporins are potent inhibitors of other biliary tract transporters [84].

As *mdr1a* knock-out mice with a disrupted blood–brain barrier are highly susceptible to neurotoxins [97], perhaps CNS toxicity could be expected with potent P-glycoprotein inhibitors. However, for the knock-out mice, all was well until they were exposed to pesticide ivermethacin [97] and thus, unless the chemotherapy drugs are neurotoxic, perhaps this will not occur. Trials with highly potent P-glycoprotein inhibitors may throw light on this area of speculation. Furthermore, the knock-out mice had decreased clearance of vinblastine [97], so it may be inevitable that highly effective inhibitors of P-glycoprotein will alter natural product cytotoxic pharmacokinetics.

The possibility of modulating P-glycoprotein-mediated drug resistance illustrates the complexities of applying promising laboratory findings in the clinical setting. In the early years, pragmatism determined which drugs (such as tamoxifen, quinidine and verapamil) entered clinical trial. Although there were some early clinical successes in lymphoma with verapamil [18], the multitude of apparently negative data in solid tumours led to pessimism [98].

However, the realisation of the fundamental importance of utilising the information from pharmacokinetics [99] combined with the development of drugs, such as PSC 833, CGP 41251 and GG918, offer opportunities to improve the treatment of solid tumours. The clinical development of the most recently discovered and potent P-glycoprotein inhibitors has benefited greatly from the knowledge gleaned from early modulation trials. Current preclinical and subsequent clinical development is depicted in an idealised scheme in Figure 4.

The other factor which will drive this is the activity of new cytotoxic drugs, such as paclitaxel in ovarian cancer [100], a tumour which frequently expresses P-glycoprotein in relapse [101].

The largest clinical benefit from P-glycoprotein modulation is unlikely to be seen in advanced relapsed cancers after multiple regimens of chemotherapy. This is because these tumours are likely to be clonally heterogeneous and express more than one mechanism of resistance. However, as experimental work using the tool of fluctuation analysis tells us, mutations which give rise to P-glycoprotein MDR are relatively common [10]. It, therefore, seems likely that a neoadjuvant modulation strategy is worth clinical exploration, and more likely to be the defining test of the value of P-glycoprotein inhibition. Such randomised phase III trials need extensive preparation to ensure that the chosen modulators do not confound the trial interpretation by altering the AUC of a key cytotoxic drug. Furthermore, it will be essential to

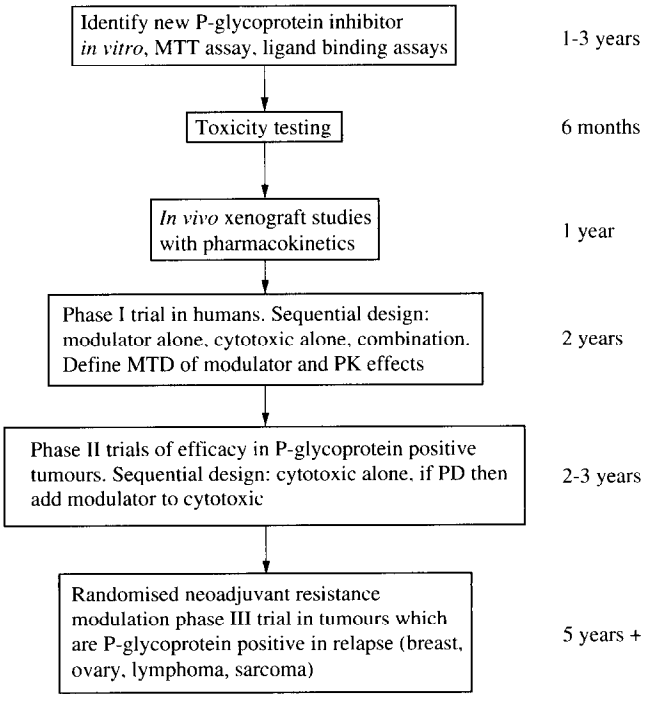


Figure 4. Outline development of P-glycoprotein inhibitors. PK, pharmacokinetics; PD, progressive disease; MTD, maximum tolerated dose; MTT, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide.

attempt to tissue type the relapse cases and determine whether this strategy can eliminate P-glycoprotein expressing clones.

The story of P-glycoprotein illustrates the difficulty of translating exciting laboratory findings into meaningful clinical trials. Indeed, we may have reached a point where modulation trials can progress meaningfully into the phase II setting with potent P-glycoprotein inhibitors in the next few years. Beyond that lies many years of conducting randomised trials in various diseases with neoadjuvant modulation with pharmacokinetically adjusted chemotherapy doses. This will allow the true effect of P-glycoprotein inhibition to be isolated and quantified.

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