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A phase I and pharmacologic study of the MDR converter GF120918 in combination with doxorubicin in patients with advanced solid tumors

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Abstract *Background:* Resistance to chemotherapy can partly be explained by the activity of membrane bound P-glycoprotein. Competitive inhibition of P-glycoprotein, by multidrug resistance (MDR) converters, may overcome this MDR. Previously studied MDR converters either have serious intrinsic side effects or considerably influence the pharmacokinetics of cytotoxic agents at concentrations theoretically required to convert MDR. GF120918 is a third-generation MDR converter with high affinity for P-glycoprotein and can be given orally. We performed a phase 1 study with escalating doses of GF120918 in combination with doxorubicin. *Patients and methods:* The study group comprised 46 patients with advanced solid tumors. Doxorubicin was administered on day 1 (cycle 1), GF120918 on days 22–24 (cycle 2), and on days 29–33 with doxorubicin

administered on day 31 (cycle 3). Pharmacokinetics of both GF120918 and doxorubicin were studied. The starting daily dose of GF120918 was 50 mg and was to be increased in subsequent cohorts until a steady state plasma level of 100 ng/ml was reached. The starting dose of doxorubicin was 50 mg/m² and was to be increased after reaching the target dose level of GF120918. *Results:* In 37 of the 46 patients, full pharmacokinetic data from the three scheduled cycles were obtained. Pharmacokinetics of GF120918 showed a less than linear increase in C_{max} with increasing doses, with considerable interpatient variation. The target steady-state plasma level for GF120918 was exceeded in 12 out of 19 patients who received 400 mg GF120918 alone twice daily and in 12 of 17 patients who received 400 mg GF120918 twice daily in combination with doxorubicin. GF120918 pharmacokinetics were not influenced by co-administration of doxorubicin. The doxorubicin AUC was only marginally influenced by GF120918 and only at the highest dose levels. In these patients there was a significant increase in the AUC of doxorubicinol in cycle 3 as compared to cycle 1. Hematologic toxicity mainly consisted of neutropenia and was more severe in cycle 3 than in cycle 1 (13 vs 5 patients with grade 4 neutropenia, $P=0.003$). Neutropenic fever was the dose-limiting toxicity at a doxorubicin dose of 75 mg/m² with 400 mg GF120918 twice daily. The toxicity of GF120918 was limited to somnolence in eight patients and occasional gastrointestinal complaints. *Conclusion:* GF120918 is an MDR converter with only minimal side effects at a dose level yielding concentrations able to convert the action of P-glycoprotein in vitro. A doxorubicin dose of 60 mg/m² on day 3 in combination with 400 mg GF120918 twice daily on days 1–5 is an acceptable regimen for further clinical trials.

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Introduction

Intrinsic or acquired resistance to cytotoxic drugs remains a major obstacle in the attempts to improve treatment results in advanced cancer. One of the mechanisms of drug resistance involves the overexpression of the multidrug resistance (MDR) gene-1 encoding the membrane-bound transporter P-glycoprotein (Pgp) [8, 12, 19, 20, 23]. Pgp acts as an ATP-dependent efflux pump resulting in a lower intracytoplasmic concentration of cytotoxins in Pgp-overexpressing cells as compared to sensitive cells. By this mechanism, resistance to a variety of naturally occurring cytotoxic drugs such as anthracyclines, anthracenediones, epipodophyllotoxins, vinca-alkaloids and taxanes can, in part, be explained. MDR-related chemotherapy resistance is common in renal, colon, hepatocellular, pancreatic and breast cancer, and can develop rapidly after exposure to chemotherapy of initially sensitive tumors such as ovarian cancer and small-cell lung cancer. In leukemias, non-Hodgkin lymphomas, soft tissue sarcomas and high-grade osteosarcomas, MDR-overexpression at diagnosis is correlated with a decreased survival [1, 3, 13, 16, 21].

Several drugs competitively inhibit the action of the Pgp efflux pump, thereby increasing the intracellular concentration of cytotoxic agents. Verapamil, cyclosporin and quinidine are the most extensively studied of these so-called MDR converters [7, 24]. Clinical use at serum concentrations able to reverse Pgp activity in vitro has been hampered by either significant side effects of the MDR converter itself or by increased toxicity of the cytotoxic agent used. Only in hematologic malignancies has the addition of some of the above-mentioned MDR converters to anthracycline-containing therapy shown benefit [2, 4, 22, 26, 27, 33, 34].

GF120918 (9,10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-4-acridine-carboxamide hydrochloride; Fig. 1) is a third-generation MDR converter [14]. The drug itself has no antitumor activity and has the advantage that it can be administered orally. In *in vitro* studies the IC_{50} of doxorubicin in the Pgp-positive ovarian cancer cell line SKV1B was decreased 140-fold by GF120918 which was more potent than verapamil in these cell lines. In *in vivo* studies GF120918 potentiated the activity of doxorubicin 60-fold in the P388/Dox leukemia model and two- to threefold in the C26 colon carcinoma model. In doxorubicin-resistant cell lines CH^R C5 and OV1/

DXR cells were maximally sensitized to doxorubicin at a concentration of 100 ng/ml [14].

In toxicology studies in the dog the drug was well tolerated. Toxic changes observed at doses > 25 mg/kg per day were Kupffer cell hypertrophy, multifocal and reversible hepatocellular necrosis, gastric parietal cell degeneration, mesenteric lymph node histiocytosis and subacute perivasculitis. At the highest dose studied of 500 mg/kg per day, ophthalmologic studies revealed multiple gray foci in the tapetum lucidum of the eye. All changes were reversible after stopping the drug.

In human volunteers the pharmacokinetic parameters of GF120918 have been studied after single oral doses of 5–80 mg in the fasting and the fed state. The oral bioavailability is 40–50% with a better absorption when GF120918 is administered after a meal. The plasma half life is 10–15 h (data from file Glaxo Wellcome Research). We performed a phase 1 study of increasing doses of GF120918 in combination with doxorubicin. Based on data from studies with other MDR converters showing increased doxorubicin toxicity, the selected starting dose of doxorubicin was 50 mg/m². The GF120918 starting dose of 50 mg once daily was based on pharmacokinetic studies in human volunteers and the dose was increased in subsequent patient cohorts until a target steady-state plasma concentration of 100 ng/ml was reached, after which further dose escalation of doxorubicin was planned.

Patients and methods

Patients

To be eligible for the study patients were required to have a metastatic solid tumor, histologically or cytologically proven, not amenable to curative surgery or radiotherapy. Eligibility criteria further included: age > 18 years, a World Health Organization (WHO) performance status 0–2, a life expectancy > 3 months, measurable or evaluable lesions at physical examination or on CT scan/MRI, off previous chemotherapy for at least 3 weeks (6 weeks in case of mitomycin C or nitrosoureas), no prior treatment with anthracyclines or anthracenediones, white blood cell count (WBC) $> 3.0 \times 10^9/l$, granulocytes (ANC) $> 1.5 \times 10^9/l$, platelets $> 120 \times 10^9/l$, prothrombin time (PT) and partial thromboplastin time (PPT) less than 1.3 times the control value, bilirubin $< 20 \mu\text{mol/l}$, AST and ALT less than three times the upper limit of normal, serum creatinine $< 120 \mu\text{mol/l}$ or creatinine clearance $> 60 \text{ ml/min}$, no documented active peptic ulcer or pancreatitis within the past 6 months, no signs of peripheral neuropathy, no signs of congestive heart failure or myocardial ischemia, a left ventricular ejection fraction (LVEF) $> 50\%$ as measured by MUGA-scan and/or no active heart disease requiring antiarrhythmics. Women of childbearing potential were required to have a negative pregnancy test and to use adequate contraception.

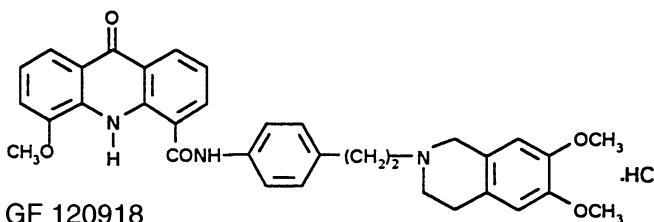


Fig. 1 Chemical structure of GF120918

Excluded from the study were patients with concomitant medical or psychologic disorders making them unsuitable for treatment or follow-up per protocol and patients with known seropositivity for HIV or with uncontrolled infections.

Screening studies included a full blood count including WBC plus differential, serum chemistry including electrolytes, creatinine, urea, bilirubin, total protein, albumin, uric acid, glucose, AST, ALT, alkaline phosphatase, LDH, activated PTT (APTT) and PT. Baseline urinalysis included tests on glucose, protein and a sediment.

All patients had a 12-lead ECG and an ophthalmologic examination by an ophthalmologist including retina photography, tests with Ishihara color plates and a Sentinel test. All screening procedures, including CT scan or MRI had to be performed within 14 days of the start of treatment.

During treatment patients had weekly a physical examination and grading of toxicities; hematologic counts were taken twice weekly, biochemistry weekly. A 12-lead ECG was obtained prior to dosing on day 1 and within 8 h of dosing on days 1, 22 and 29. ECG was repeated thereafter every second cycle. LVEF, ophthalmologic examination and CT scan or MRI were repeated after every second doxorubicin administration. The protocol was approved by the Medical Ethical Committee of the Daniel den Hoed Cancer Center. All patients signed a written informed consent before study entry.

Study design

On day 1 (cycle 1) doxorubicin was administered as a 5-min bolus infusion. On days 22–24 (cycle 2) GF120918 was administered for estimation of pharmacokinetics. GF120918 dosing was repeated from day 29 to day 33 with doxorubicin administered on day 31 (cycle 3) simultaneously with the morning dose of GF120918.

The starting dose of GF120918 was 50 mg once daily in the first cohort of three evaluable patients. After full analysis of toxicity data of the previous cohort the GF120918 dose was further escalated until a steady-state plasma concentration of 100 ng/ml was reached. Thereafter the dose of doxorubicin was escalated. The dose escalation schedules of GF120918 and doxorubicin are shown in Table 2.

If a dose-limiting toxicity (DLT) was encountered, the cohort of patients could be extended to six patients or until two patients at that dose level experienced DLT, whichever came first. DLT was defined as: an absolute neutrophil count $<0.5 \times 10^9/l$ or platelet count $<25 \times 10^9/l$ for more than 5 days or a neutrophil count $<0.5 \times 10^9/l$ with fever requiring antibiotics and/or nonhematologic toxicity of CTC grade 3 or more, in more than one-third of the patients, thus maximally in two out of six patients. An additional criterion for DLT was a decrease in the

LVEF of $>15\%$ from the baseline LVEF. The maximal tolerated dose (MTD) was defined as one dose level below the dose inducing DLT. GF120918 was supplied as tablets of 25 or 100 mg and was administered orally with 50–100 ml of water 1 h after a meal. Antacids were prohibited for 2 h before and 2 h after the intake of GF120918.

Tumor response was measured for the first time 3 weeks after cycle 3. In case of treatment benefit after cycle 3 patients could continue treatment with the doxorubicin–GF120918 combination as given in cycle 3. Further evaluation of tumor response was done after every second administration of the doxorubicin–GF120918 combination.

Pharmacokinetic studies

Details of the pharmacokinetic studies have been reported previously [28]. In brief, all blood samples were immediately centrifuged for 5 min at 3000 g to yield plasma, which was stored in polypropylene vials at -80°C until analysis. For doxorubicin, 2 ml plasma samples were obtained on days 1 and 31 at time 0 (predose) and at 5, 30 and 60 min and 2, 4, 7, 12, 24 and 48 h after dosing. After thawing, quantitative extraction was achieved by a single precipitation step of 1-ml samples with 500 μl acetone in the presence of 100 μl aqueous zinc sulfate. Concentrations of doxorubicin and its metabolite doxorubicinol were determined by a new reversed-phase HPLC method with a lower limit of detection of 500 pg/ml for both compounds [5].

For GF120918, 5 ml samples were obtained on days 22 and 29 at time 0 (predose) and at 1, 2, 4, 7, 12 and 24 h after dosing and on day 31 (in combination with doxorubicin) at time 0 and at 4 h after dosing on days 31 and 32 and 6 h after dosing on day 32. Plasma samples and internal standard were alkalinized and extracted with methyl *t*-butyl ether. Analysis was performed using a C18 HPLC column with fluorescence detection. The assay was validated over a concentration range of 0.5–500 ng/ml.

Pharmacodynamic studies

The intracellular accumulation of the fluorescent probe rhodamine 123 (Rh123) in $\text{CD56}^+/\text{CD3}^-$ lymphocytes was determined as a surrogate marker for the functional capacity of the Pgp pump activity in the presence or absence of GF120918 [35]. $\text{CD56}^+/\text{CD3}^-$ cells are natural killer cells which have a high content of Pgp. The test was performed on day 22 before (T0) and 4 h after (T4) administration of GF120918. This analysis was repeated 1 week later with samples drawn during treatment with GF120918 on day 29 and with GF120918 plus doxorubicin on day 31.

Whole blood (100 μl) was incubated with Rh123, PERCP-conjugated anti-CD3 monoclonal antibody

Table 1 Patient characteristics

No. of patients entered	46
No. of patients fully evaluable	37
Gender	
Male	26
Female	20
Age (years)	
Median	51
Range	25–78
WHO performance status	
0	30
1	15
2	1
Primary tumor	
Colorectal	13
Ovarian	8
Renal	6
Melanoma	3
Esophagus	3
Uterine cervix	2
Lung	2
Mesothelioma	2
Sarcoma	2
Pancreas	1
ACUP	2
Bladder	1
Hepatocellular carcinoma	1
Prior treatment	
Surgery	35
Radiotherapy	10
Chemotherapy	37

(Becton Dickinson, San Jose, Calif.) and PE-conjugated anti-CD56 monoclonal antibody (Becton Dickinson). After lysing the cells with cold orthoimmune lysing solution for 10–15 min the cells were centrifuged and the pellet was resuspended in phosphate-buffered saline with 10% fetal calf serum and kept on ice in the dark until analyzed on a FACScan flow cytometer (Becton Dickinson). The results are expressed as the ratio of the mean fluorescence intensity of Rh123 in the sample obtained at 4 h (T4) vs that in the predose sample (T0) on days 22, 29 and 31. Also the relationship between the percentage shift in fluorescence and plasma GF120918 concentration at 4 h after dosing on days 22, 29 and 31 was examined.

The response was determined as:

$$\% \text{ shift in fluorescence} = \frac{\text{fluorescence at } 4 \text{ h} - \text{fluorescence at } 0 \text{ h} \times 100}{\text{fluorescence at } 0 \text{ h}}$$

Statistics

To test toxicity differences between treatment cycle 3 and cycle 1, McNemar's test for correlated proportions was used. Pharmacologic differences between cycle 3 and cycle 1 were tested with the two-sided Student *t*-test. The correlation between the peak plasma levels of GF120918 and the AUC of doxorubicin and doxorubicinol and their cycle 3/1 ratio were analyzed by means of Spearman's correlation coefficient. *P* values < 0.05 were considered as statistically significant. All statistical calculations were performed using NCSS (version 5.X; Dr J. Hintze, Kayesville, Utah) and STATGRAPHICS plus (version 2; Manugistics, Rockville, Md.).

Results

Patient characteristics

Entered into the study were 46 patients of whom 37 completed cycles 1–3. All patients were eligible. Reasons not to complete the first three scheduled cycles were early progressive disease in seven patients and a bleeding duodenal ulcer and a pulmonary embolism in one patient each. The patient characteristics are presented in detail in Table 1. Table 2 presents the dose levels of doxorubicin and GF120918 studied, as well as the number of patients treated per cohort. In total 132 cycles of doxorubicin were administered, of which 86 were in combination with GF120918, for a median of two doxorubicin cycles per patient (range 1–8).

Toxicity

The hematologic toxicity mainly consisted of leukocytopenia and granulocytopenia, which were more severe

Table 2 Dose levels of GF120918 and doxorubicin administered and related hematologic toxicity

Dose level	GF120918		Doxorubicin (mg/m ²)	No. of patients entered	No. of patients evaluable ^a	No. of patients with ANC grade 4 (cycle 1/cycle 3)	ANC nadir median (range) (×10 ⁹ /l)	
	Dose (mg)	Frequency					Cycle 1	Cycle 3
1	50	Daily	50	6	5	0/0	1.03 (0.70–2.20)	1.27 (0.57–3.70)
2	50	Twice daily	50	5	5	1/3	0.70 (0.37–1.68)	0.44 (0.11–4.26)
3	100	Twice daily	50	5	5	1/0	1.38 (0.28–2.55)	0.96 (0.36–2.71)
4	200	Twice daily	50	5	5	0/3	1.31 (0.60–1.85)	0.99 (0.29–3.32)
5	400	Twice daily	50	11	7	0/0	1.30 (0.54–2.30)	0.57 (0.52–2.21)
6	400	Twice daily	60	8	5	1/2	0.67 (0.07–1.42)	0.73 (0.26–2.77)
7	400	Twice daily	75	6	5	2/5	0.51 (0.50–0.79)	0.11 (0.02–0.24)

^aPatients who completed cycles 1–3 including pharmacokinetic studies

Table 3 Non-hematologic toxicity

Side effect	GF120918 (cycle 2); 40 administrations		Doxorubicin (cycle 1); 46 administrations		Doxorubicin + GF 120918 cycle 3 and further; 86 administrations	
	Events	Frequency ^a	Events	Frequency ^a	Events	Frequency ^a
Nausea	6	0.15	29	0.63	57	0.66
Vomiting	5	0.12	19	0.41	49	0.60
Fatigue	2	0.05	14	0.30	23	0.27
Somnolence	4	0.10	0	0	19	0.22
Diarrhea	1	0.02	2	0.04	18	0.21
Mucositis	0	0	2	0.04	27	0.31
Fever	1	0.02	5	0.11	11	0.13
Dizziness	2	0.05	6	0.13	10	0.11
Anorexia	2	0.05	6	0.13	14	0.16

^aFrequency = number of events/no. of treatments in group

in cycle 3 than in cycle 1. The data are presented in Table 2. In cycle 1, 8 out of 37 patients had grade 3/4 leukocytopenia vs 20 patients in cycle 3 ($P=0.001$); five patients had a granulocytopenia grade 4 in cycle 1 vs 13 in cycle 3 ($P=0.003$). As expected, hematologic toxicity was more pronounced at the higher doses of doxorubicin. The DLT was neutropenic fever occurring in three out of five patients at the highest dose level studied (doxorubicin 75 mg/m^2 + GF120918 400 mg twice daily). Thrombocytopenia grade 3 or 4 was not observed in cycle 1 but was observed in one patient each during cycle 3. There were no deaths due to toxicity. The data on nonhematologic toxicity are summarized in Table 3. To make a comparison easier between single-agent doxorubicin vs the combination with GF120918, the nonhematologic toxicities are presented as the number of toxic events, divided by the number of cycles administered. With the exception of mucositis grade 3 reported in two patients at the highest dose level, none of the nonhematologic toxicities exceeded grade 2. Vomiting, diarrhea and mucositis were slightly more frequently reported with the combination treatment than with single-agent doxorubicin but none of these reached statistical significance. Somnolence was a side effect of GF120918 as it was never reported after single-agent doxorubicin but was reported after single-agent GF120918 and in the combination cycles 19 times by a total of 8 patients. The occurrence of somnolence did not appear to be related to GF120918 dose. Other side effects of GF120918 were minimal with only nausea and vomiting reported incidentally.

In eight out of 16 patients who continued treatment with a cumulative doxorubicin dose $>200 \text{ mg/m}^2$, the LVEF decreased $>10\%$; one of the patients required diuretic therapy, the others developed no signs of cardiac failure. In five patients the decrease in LVEF was the reason for stopping treatment during ongoing stable disease. ECG changes were not observed. Follow-up ophthalmologic examinations were unremarkable with the exception of the development of cotton-wool exudates in one patient.

Responses

Response was evaluable in 36 patients. One patient with liver metastases from an adenocarcinoma of unknown primary had a partial response lasting 11 months. Stable disease was seen in 19 patients with a median time to progressive disease of 4 months (range 3–8 months).

Pharmacokinetics

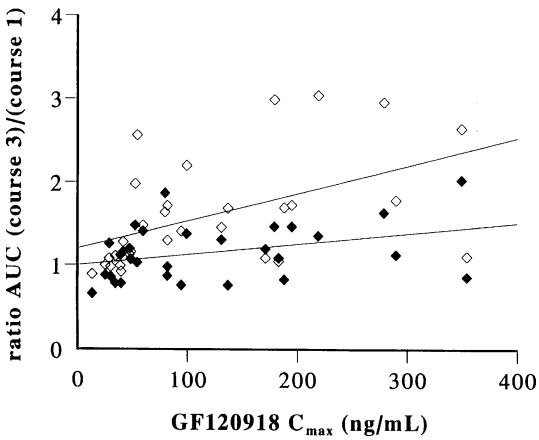
Pharmacokinetic data are presented in Table 4 for GF120918 and in Table 5 for doxorubicin and doxorubicinol. GF120918 C_{\max} showed a high interpatient variability; the increase in C_{\max} with dose was less than linear. The $C_{ss,avg}$ aimed for was exceeded in 12 out of 19 patients at GF120918 doses of 400 mg twice daily alone and in 12 out of 17 patients with GF120918 400 mg twice daily in combination with doxorubicin. Coadministration of doxorubicin in cycle 3

Table 4 Pharmacokinetic studies of GF120918, cycle 2 vs cycle 3. The data are presented as median (range)

Dose level	Doxorubicin (mg/m ²)	GF120918 (mg)	Cycle 2 (GF120918 alone)			Cycle 3 (GF120918 with doxorubicin)		
			n	$C_{ss,avg}$ (ng/ml)	C_{\max} (ng/ml)	n	$C_{ss,avg}$ (ng/ml)	C_{\max} (ng/ml)
1	50	50 daily	6	17.6 (12.6–27.3)	31.1 (21.6–55.5)	5	19.5 (10.4–51.4)	34.2 (13.2–97.4)
2	50	50 twice daily	5	36.1 (29.1–102.3)	49.4 (38.2–148.9)	5	34.3 (31.6–127.5)	54.0 (41.4–187.6)
3	50	100 twice daily	5	31.5 (21.7–95.2)	57.5 (29.8–113.7)	5	24.2 (18.5–65.5)	39.5 (25.0–94.5)
4	50	200 twice daily	5	65.1 (42.7–101.8)	96.8 (57.6–133.1)	5	48.4 (34.7–141.1)	79.3 (48.3–171.0)
5	50	400 twice daily	8	130.4 (64.6–203.9)	162.9 (90.6–261.2)	7	103.7 (61.7–258.2)	137.1 (100.5–290.0)
6	60	400 twice daily	7	120.9 (71.5–304.4)	161.4 (105.3–336.5)	5	128.5 (49.6–253.5)	195.4 (81.7–354.1)
7	75	400 twice daily	4	147.9 (68.7–259.9)	184.4 (84.3–383.9)	5	156.7 (47.5–235.9)	177.0 (59.3–349.1)

Table 5 Pharmacokinetics of doxorubicin and doxorubicinol, cycle 1 vs cycle 3 (cycle 1 doxorubicin single agent, cycle 3 doxorubicin + GF120918)

Dose level	GF120918 (mg)	Doxorubicin (mg/m ²)	No. of patients	Doxorubicin AUC _{0-∞} (ng h/ml)			Doxorubicinol AUC _{0-∞} (ng h/ml)			Cycle 1/3 AUC ratio	
				Cycle	Median	Range	% CV	Median	Range	% CV	Doxorubicin
1	50 daily	50	1	5	1,700	1,032–2,006	21.1	483	213–1,159	61.4	0.86
			3	5	1,419	1,271–2,252	23.3	471	342–1,443	49.3	0.99
2	50 twice daily	50	1	5	1,217	934–2,071	31.1	395	211–718	42.1	1.03
			3	5	1,192	1,009–1,805	21.5	672	239–1,232	45.9	1.70
3	100 twice daily	50	1	5	1,395	1,177–2,748	34.1	443	349–744	26.9	0.88
			3	5	1,743	1,039–2,090	21.7	555	402–1,049	35.9	1.08
4	200 twice daily	50	1	5	1,024	775–1,315	23.5	460	245–721	30.0	1.23
			3	5	1,532	1,137–2,131	22.4	580	367–1,182	43.6	1.47
5	400 twice daily	50	1	7	1,143	921–1,927	23.5	420	318–1,276	49.6	1.20
			3	7 ^a	1,501	787–2,183	18.8	1,034	612–2,288	48.0	1.94
6	400 twice daily	60	1	8	1,836	1,227–2,193	13.2	742	517–859	7.8	1.20
			3	4	2,038	1,637–2,236	11.7	890	724–1,371	25.2	1.04
7	400 twice daily	75	1	6	1,716	937–3,022	23.4	676	403–1,097	24.8	2.99
			3	5	2,502	2,255–3,056	11.5	1,410	1,089–3,377	43.2	

^aData on doxorubicinol at this dose level, 6 patients studied.**Fig. 2** Ratio of the AUC of doxorubicin (filled diamonds) and doxorubicinol (open diamonds) of cycle 3 vs cycle 1 as compared to the C_{max} of GF120918

did not appear to influence the pharmacokinetics of GF120918.

Analysis of the AUC of doxorubicin and doxorubicinol showed that the AUC of doxorubicinol was higher with the combination treatment in cohort 5 ($P=0.049$; Student's *t*-test) and cohort 7. As the difference in cohort 5 could only be explained by two out of five patients displaying a more than threefold increase in the AUC of doxorubicinol, an analysis was performed of all AUCs of doxorubicin and doxorubicinol of cycle 3 vs cycle 1 as a function of the maximum plasma concentration of GF120918 (Fig. 2). This analysis showed that there was a minimal but significant overall increase in the AUC of doxorubicin (Pearson's $r=0.375$; $P=0.041$) but a clear significant increase in the doxorubicinol AUC (Pearson's $r=0.499$; $p=0.005$) in cycle 3 vs cycle 1. The kinetic interaction between doxorubicinol and GF120918 was more pronounced in patients with GF120918 concentrations >80 ng/ml ($P=0.011$) while in these patients there was no interaction with the AUC of doxorubicin ($P=0.328$).

Flow cytometric analysis of Rh123 accumulation in CD-56⁺ lymphocytes

Analysis of Rh123 accumulation in CD-56⁺/CD3⁻ lymphocytes obtained from the patients showed that up to a single dose of 100 mg GF120918, no effect on the T4/T0 ratio was observed on either day. This indicates that with this clinical dose of GF120918, no significant amount of Rh123 is retained by the cells. In patients treated with dosages of GF120918 of 200 mg and 400 mg, the T4/T0 ratios of Rh123 retention had increased to 1.3–1.6 and 2.6–3.0, respectively. No difference was observed between day-22 and day-29 values. However, the T4/T0 ratio in patients treated on day 31 with the highest dose of GF120918 plus doxorubicin was lower than that observed in these patients on day 22 and day 29 which can be explained by the pres-

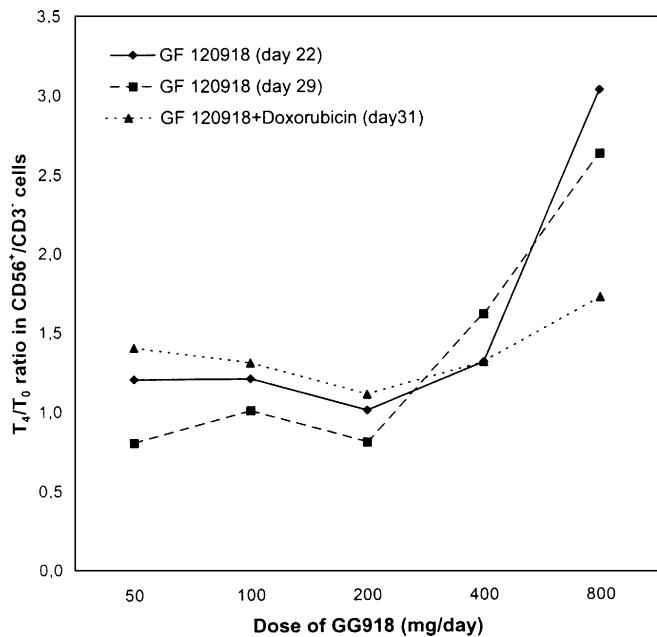


Fig. 3 Ratio of the mean fluorescence intensity of Rh123 at 4 h (T4) vs predose (T0) in $CD56^+$ / $CD3^-$ cells at the dose levels of GF120918 studied. The lower T4/T0 ratio on day 31 can be explained by the fact that at T0 GF120918 is still present in the cells

ence of GF120918 in the cells from dosages the day before (Fig. 3).

The relationship between the percentage shift in fluorescence and plasma concentration of GF120918 after the first dose is shown in Fig. 4. There was variability within the data, but the percentage shift in fluorescence clearly increased with increasing plasma GF120918 concentrations. A maximal effect was apparently not achieved in this study, making the fitting of an E_{max} or sigmoidal E_{max} model impossible.

Discussion

Clinical studies with MDR converters to overcome drug resistance have only to some extent been successful in

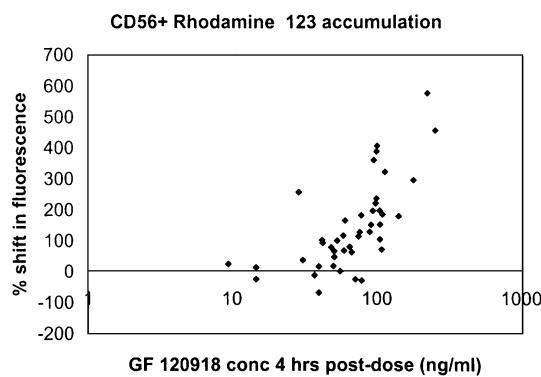


Fig. 4 Effect of GF120918 on the Rh123 accumulation in $CD56^+$ / $CD3^-$ cells expressed as the percentage shift in fluorescence

hematologic malignancies using doxorubicin or mitoxantrone combinations in non-Hodgkin's lymphoma, acute leukemia or multiple myeloma [4, 25, 26]. In solid tumors studies have as yet failed to show any benefit from adding an MDR converter which has even resulted in increased toxicity, either from the MDR converter itself or from an increase in the toxicity of the cytotoxic drug administered. Verapamil, for instance, in several studies resulted in considerable cardiovascular side effects such as hypotension, first degree and total heart block and congestive heart failure at plasma concentrations below that expected to achieve Pgp inhibition [4, 21]. The increased toxicity of doxorubicin in combinations with verapamil or cyclosporin could be explained pharmacokinetically by an increase in the AUC of doxorubicin, a higher C_{max} and a lower systemic clearance [15]. With coadministration of an MDR converter, similar interactions have also been shown between etoposide and cyclosporin [17]. Another side effect of cyclosporin is hyperbilirubinemia, possibly because of inhibition of a bilirubin transporter in bile canaliculi interfering with drug excretion [17].

In recent studies, second- or third-generation MDR converters that show a higher affinity for Pgp such as dextroverapamil, the *R*-isomer of verapamil, and the cyclosporin analogue PSC-833, have been investigated. Although these newer MDR converters appear to have less intrinsic toxicity than the parent compounds, these drugs also decrease the systemic clearance of the cytotoxic agents used leading to more profound toxicity necessitating dose reduction of the cytotoxic agent or a change in the mode of administration from bolus to a less-convenient continuous infusion [6, 9, 31].

Other more recently developed MDR converters are also not devoid of side effects. The triazineaminopiperidine derivative, S9788, in combination with doxorubicin, causes conduction disturbances resulting in AV block, ventricular arrhythmias and torsades de pointes [29], while zosuquidar.3HCl trihydrochloride (LY335979) causes significant reversible cerebellar ataxia [24].

In the present study we explored the feasibility of the combination of the MDR converter GF120918 and doxorubicin. In preclinical studies, GF120918 has shown a high affinity for Pgp and is able to block Pgp at concentrations as low as 30 ng/ml. In human male volunteers, single doses up to 80 mg are well tolerated with mild headache, dizziness and somnolence as the most frequently reported side effects. Pharmacokinetic studies in the volunteers have shown a dose-dependent linear increase in the C_{max} of GF120918. In the present study GF120918 showed no side effects apart from mild somnolence and slight gastrointestinal complaints. Only at the target dose of 400 mg twice daily and at plasma levels > 80 ng/ml was a pharmacologic interaction seen for the metabolite doxorubicinol but not for doxorubicin. Pharmacodynamically, an effect of GF120918 on Rh123 retention in autologous $CD56^+$ / $CD3^-$ natural killer cells was observed at the highest

dose of 400 mg twice daily. At this dose Rh123 retention increased 2.6- to 3.0-fold as compared with initial values. On day 31 the presence of doxorubicin(ol) in the whole-blood samples of these patients resulted in a lower Rh123 retention than on day 29. This can be explained by the presence of GF120918 in the cells from previous dosing resulting in a lower ratio. Clinically the interaction caused significantly more myelosuppression, resulting in more leukocytopenia and granulocytopenia at dose levels of 100 mg twice daily or higher. Despite the increased hematologic toxicity, we were able to escalate the dose of doxorubicin to a dose commonly used as a single agent. In general the treatment was well tolerated although mucositis and vomiting were slightly more frequently reported with the combination.

Neutropenic fever was found to be the dose-limiting side effect of doxorubicin at 75 mg/m² in combination with 400 mg GF120918 twice daily. The combination of doxorubicin with GF120918, however, showed no significant antitumor activity as only one patient showed a partial response. This is in line with the disappointing results of other studies with MDR converters in solid tumors and may reflect an unfavorable selection of patients.

The increase in the AUC of doxorubicinol at the optimal dose of GF120918 may be a matter of concern for future studies with MDR converters, since anthracycline metabolites may have a more deleterious influence on the heart than the parent compound [18]. In our study, 8 out of 16 patients continuing treatment with doxorubicin beyond a total dose of 200 mg/m² showed a decrease in the LVEF of >10%. This figure is difficult to interpret as sound comparable data are lacking. In cardioprotection studies, LVEF values are seldom reported for this low cumulative doxorubicin dose [28, 30]. Gianni et al. evaluated prospectively the change in LVEF, measured by ultrasound, in breast cancer patients treated with the combination of paclitaxel and doxorubicin. Even at a cumulative doxorubicin dose of 180 mg/m², a small but significant decline in LVEF compared to the baseline was observed [10]. The observed decrease in LVEF in our patients therefore may be within the range that can be expected from doxorubicin alone. Gonzalez et al. reported a study on doxorubicin and doxorubicin metabolites in serum and tissues of mice treated with doxorubicin with or without PSC833. Although serum levels of doxorubicin and doxorubicinol were not influenced by PSC833, the tissue levels of doxorubicin and doxorubicinol were significantly higher with the combination than with doxorubicin alone. High levels were found in the intestine, liver, adrenals and kidneys. Also in the heart the difference was statistically significant albeit smaller [11]. Therefore, although our data shows only a minor influence of GF120918 on the doxorubicin pharmacokinetics, more toxicity at the tissue level might be expected. Recently a pharmacologic study in mice lacking the mdr-1a gene mediating Pgp showed a longer

retention of doxorubicin and doxorubicinol in heart tissue as compared to wild-type mice [32]. These observations should alert clinicians that a blockade of endogenous Pgp might indeed predispose to cardiac damage and that careful monitoring of cardiac function is advisable.

In conclusion, The MDR converter GF120918 can achieve plasma concentrations that inhibit Pgp both in vitro and ex vivo with minimal side effects. At the recommended dose level of doxorubicin 60 mg/m² on day 3 in combination with GF120918 on days 1–5, there exists a pharmacologic interaction between the two drugs resulting in increased hematologic toxicity. Doxorubicin can however, in contrast to combinations with other MDR converters, be administered at a dose considered clinically active in most tumor types.

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