Population pharmacokinetic and pharmacodynamic analysis to support treatment optimization of combination chemotherapy with indisulam and carboplatin

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Chemotherapy with indisulam in combination with a standard dose of carboplatin was not well tolerated in a 3-weekly regimen in a Phase I dose escalation study.
- Myelosuppression was the dose limiting toxicity.
- This pharmacokinetic–pharmacodynamic (PK–PD) study was performed to suggest a dosing regimen for the combination therapy indisulam–carboplatin that is well tolerated in patients.

WHAT THIS STUDY ADDS

- This PK–PD study supports the selection of indisulam 500 mg m⁻² and a dose of carboplatin to achieve an AUC of 6 mg min⁻¹ ml⁻¹ in a 4-weekly regimen as the recommended dose for future studies.

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AIMS

Indisulam and carboplatin have shown synergistic activity in preclinical studies. In a dose escalation study of the combination, a treatment delay was frequently required in a 3-weekly regimen to allow recovery from myelosuppression from previous cycles. A 4-weekly regimen was better tolerated, but had a decreased dose-intensity which may compromise efficacy. The aims of this study were (i) to develop a pharmacokinetic–pharmacodynamic (PK–PD) model to describe the myelosuppressive effect of the combination, and (ii) to use this model to select a dosing regimen for Phase II evaluation.

METHODS

Sixteen patients were treated at four different dose levels of indisulam (1-h infusion on day 1) and carboplatin (30-min infusion on day 2). Pharmacokinetic data were analysed with nonlinear mixed effects modelling. A semiphenological model describing chemotherapy-induced myelosuppression characterized the relationship between the pharmacokinetics and the haematological toxicity of indisulam and carboplatin. A simulation study was performed to evaluate the tolerability and dose-intensity for 3-weekly and 4-weekly treatment regimens.

RESULTS

The PK–PD model described the pharmacokinetics and the myelosuppressive effect of indisulam and carboplatin. The risk of a treatment delay at cycle 2 due to myelosuppression was unacceptably high (34–65%) in a 3-weekly regimen for various dose levels (350–600 mg m⁻² indisulam in combination with carboplatin to achieve an AUC of 4–6 mg min⁻¹ ml⁻¹). This risk was acceptable for a 4-weekly regimen (9–24%), which is in line with the clinical study results.

CONCLUSIONS

This PK–PD study supports the selection of indisulam 500 mg m⁻² and a dose of carboplatin to achieve an AUC of 6 mg min⁻¹ ml⁻¹ in a 4-weekly regimen as the recommended dose for future studies.

Introduction

Indisulam is a sulphonamide anticancer agent that inhibits the cell cycle at multiple check-points. The compound has been shown to suppress the expression of cyclin E and phosphorylation of cyclin-dependent kinase 2, causing disruption of the G₁/S transition of the cell cycle [1].

Indisulam was well tolerated in a single-agent Phase II study in patients with non-small cell lung cancer (NSCLC) pretreated with platinum-based therapy [2]. Flow cytometric analysis of bronchial brushings demonstrated that tumour-specific apoptosis was induced by treatment with indisulam, but only minor responses were observed.

In a human xenograft model for NSCLC (Lu99) in mice, indisulam and cisplatin had synergistic antitumour effects. The synergism was observed only when indisulam was administered prior to cisplatin [3]. The synergism between these drugs and the relevance of the administration sequence may be explained by indisulam-induced reduction of intracellular levels of glutathione. The expression of glutathione synthetase mRNA was downregulated 24 h after administration of indisulam [4]. Reduction of intracellular glutathione may enhance the activity of platinum-based anticancer agents, because platinum agents are intracellularly detoxified by a reaction with the thiol group of glutathione [5, 6].

Based on the synergistic antitumour efficacy of indisulam and platinum agents in preclinical studies, a Phase I dose escalation study was performed to select a recommended dose of the combination indisulam and carboplatin in a 3-weekly regimen for Phase II evaluation [4]. As expected, the dose-limiting toxicity (DLT) of the combination was myelosuppression. Both neutropenia (dose-limiting for indisulam monotherapy) and thrombocytopenia (dose-limiting for carboplatin monotherapy) were frequently observed [7, 8]. Indisulam in combination with a standard dose of carboplatin [area under the concentration–time curve (AUC) 5–6 mg min⁻¹ ml⁻¹] was not well tolerated in a 3-weekly regimen, because treatment delays were often required for patients to recover from serious myelosuppression [4]. The combination therapy did not seem feasible in a 3-weekly regimen for further Phase II evaluation. At the dose level 500 mg m⁻² indisulam and 6 mg min⁻¹ ml⁻¹ carboplatin, no DLTs were observed, and a second treatment cycle could be administered 4 weeks after the first cycle. Therefore, the investigators suggested that a 4-weekly dosing regimen of 500 mg m⁻² indisulam and 6 mg min⁻¹ ml⁻¹ carboplatin should be considered for Phase II development. However, in the clinical study only a limited number of patients were treated at the different dose levels and treatment intervals.

Platinum-based doublet chemotherapy has long been the standard treatment for patients with advanced NSCLC [9]. Most regimens are administered in 3- or 4-weekly treatment cycles, allowing time for bone marrow recovery

[10–12]. To increase dose intensity and to optimize the efficacy of platinum-based combination treatments, weekly and biweekly regimens are also being evaluated [13–15]. For the combination indisulam–carboplatin, the extension of each treatment cycle to 4 weeks may compromise dose intensity and efficacy during Phase II evaluation.

In this study, a pharmacokinetic and pharmacodynamic (PK–PD) analysis was performed to evaluate the contribution of both compounds to the severe myelosuppression observed in the clinical study. The safety and tolerability of combination therapy with indisulam and carboplatin were evaluated *in silico* for various dose levels in 3-weekly and 4-weekly treatment schedules using PK–PD modelling. A treatment regimen was considered safe and tolerable if the risk of DLT and/or treatment delay was <33%. This was in accordance with the commonly used definition in Phase I studies of the non-tolerated dose level (i.e. the lowest dose level where two or more out of six patients experience DLT). The aim of this analysis was to select a tolerable dosing schedule with maximal dose intensity for the indisulam–carboplatin regimen for Phase II evaluation.

Methods

Clinical study design

A Phase I dose escalation study was performed in patients with solid tumours who did not respond to standard chemotherapy [4]. Indisulam was administered in a 1-h infusion on day 1 of each 3-weekly treatment cycle. The indisulam dose was based on body surface area (BSA), and escalation was foreseen from 350 to 500, 600 and 700 mg m⁻². Carboplatin doses were calculated with the Calvert formula, targeted to an AUC of 6 mg min⁻¹ ml⁻¹ [16].

Carboplatin was administered in a 30-min infusion on day 2. Patients were treated in three-patient cohorts that were extended to six-patient cohorts if any DLT was observed. When two or more DLTs were observed in a six-patient cohort, the carboplatin dose was reduced to 5 mg min⁻¹ ml⁻¹ in the subsequent cohort. If the combination of indisulam with 5 mg min⁻¹ ml⁻¹ carboplatin was tolerated, the dose of indisulam was further increased to the next dose level. Patients were assessed for toxicity and pharmacokinetics during the first treatment cycle. After the first cycle, treatment could continue, provided that patients were recovered from neutropenia (neutrophil count $\geq 1.5 \times 10^9$ l⁻¹) and thrombocytopenia (thrombocyte count $\geq 100 \times 10^9$ l⁻¹). To promote recovery from severe myelosuppression, patients could receive a thrombocyte concentrate or could be treated with granulocytes-colony stimulating factor (G-CSF) at the discretion of the investigator. The study protocol was approved by the local medical ethics committee, and all patients gave written informed consent. Details about patient evaluation and follow-up have been described in a previous publication [4].

Data collection

Pharmacokinetic sampling was performed in all patients during the first treatment cycle. For bioanalysis of indisulam in plasma, 15 blood samples (4 ml) were collected: pre-infusion, 30 min after the start of infusion, at the end of the infusion, at 10 and 30 min and at 1, 2, 4, 6, 8, 24, 48, 72, 96 and 168 h after the end of the infusion. In addition, indisulam samples were taken prior to infusion and at the end of infusion during cycle 2. The samples were centrifuged at room temperature for 10 min at 1500 *g* immediately after collection. Plasma was stored at -20°C until analysis. Indisulam plasma concentrations were measured by a validated method using high-performance liquid chromatography coupled to an electrospray ionization tandem mass spectrometer (LC/ESI-MS/MS) as described previously [17]. The accuracy, within-assay and between-assay precision of this method were 93.6–100.7%, 1.7–6.3% and 5.0–10.3%, respectively. The lower limit of quantification (LLQ) of indisulam was $0.1\text{ }\mu\text{g ml}^{-1}$.

For carboplatin analysis, eight blood samples (5 ml) were collected: before administration, at the end of infusion and at 1, 2, 4, 6, 8 and 23 h after the end of infusion. The samples were centrifuged at 4°C for 5 min at 1500 *g*, and plasma was stored at -20°C until analysis. Platinum concentrations were also measured in plasma ultrafiltrate after ultrafiltration at 1500 *g* for 15 min at room temperature using Amicon ultrafiltration devices with a YMT-14 membrane (30-kDa molecular weight cut-off; Millipore Corp., Bedford, MA, USA). Graphite-furnace atomic-absorption spectrometry was used for bioanalysis [18]. Accuracy, within-day and between-day precision of this method were 93.9–103.3%, 2.5–8.6% and 1.5–10.2%, respectively. The LLQ of platinum was $0.24\text{ }\mu\text{mol l}^{-1}$.

An extra blood sample was collected to determine the genotype for the cytochrome P450 enzymes CYP2C9 and CYP2C19, which are recognized as the major indisulam-metabolizing enzymes. DNA was isolated from peripheral lymphocytes using the Nucleon BACC kit (Amersham Life Sciences, Little Chalfont, UK) and Qiagen (Qiagen, Hilden, Germany) kits. Fluorescent allele-specific hybridization was used to determine the genotype for CYP2C9*2 and CYP2C9*3. An amplification refractory mutation system was applied for CYP2C19*3. The CYP2C19*2 mutation was detected by a real-time polymerase chain reaction method.

Blood counts of neutrophils and thrombocytes were assessed twice weekly as clinical routine measurements.

Population pharmacokinetic and pharmacodynamic model development

Indisulam pharmacokinetics, carboplatin pharmacokinetics and the myelosuppressive effect of the combination treatment were described by compartmental models using nonlinear mixed effects modelling. All data were logarithmically transformed and analysed with NONMEM (version V, level 1.1) (GloboMax LLC, Hanover, PA, USA) [19].

The First-Order Conditional Estimation (FOCE) method was used, with interaction (INTER) between the interindividual and residual random effects, to estimate the pharmacokinetic parameters of carboplatin. The First-Order (FO) method was used for estimation of the pharmacokinetic parameters of indisulam and the pharmacodynamic parameters of myelosuppression in order to restrict run times to acceptable duration.

Variability between patients was described with an exponential function ($P_i = \text{TVP} + \exp(\eta_i)$). For each i^{th} patient, the individual deviation (η_i) from the population typical value (TVP) was estimated. The difference between observations and the corresponding predictions was modelled as an additive error on a logarithmic scale ($\ln(\text{OBS}_{ij}) = \ln(\text{PRED}_{ij}) + \varepsilon_{ij}$), where ε_{ij} represents the difference between the natural logarithm of the j^{th} observation in the i^{th} patient and the corresponding prediction.

Pharmacokinetic model of indisulam A semiphysiological pharmacokinetic model of indisulam has been developed previously [20]. Briefly, the backbone of the pharmacokinetic model consisted of four physiological compartments: plasma (PL), red blood cells (RBC), interstitial fluid (IF) and tissue (TIS) (Figure 1). Indisulam was saturably bound to PL proteins in plasma and in IF ($B_{\max\text{ PL}}, K_{\text{D PL}}, B_{\max\text{ IF}}, K_{\text{D IF}}$), to carbonic anhydrase in RBC ($B_{\max\text{ RBC}}, K_{\text{D RBC}}$) and to tissue components ($B_{\max\text{ TIS}}, K_{\text{D TIS}}$). In addition, indisulam was non-specifically bound to RBC and tissue components ($N_{\text{RBC}}, N_{\text{TIS}}$). Drug elimination was described by two parallel pathways: a linear elimination pathway (CL) and a saturable Michaelis–Menten pathway ($V_{\max}, K_m$).

Data from the current study were included to extend this pharmacokinetic model to a pharmacokinetic and pharmacogenetic model. This extended model was used in the current analysis and has been published previously [21]. Mutations of the CYP2C genes were related to the pharmacokinetic parameters describing the elimination of indisulam (CL and $V_{\max}$). The CYP2C9*3 mutation was related to a reduced maximal elimination rate ($V_{\max}$) of indisulam. Polymorphisms of the CYP2C19 gene (*2 and *3) were related to a lower indisulam clearance (CL). Maximum *a posteriori* (MAP) Bayesian estimates of individual pharmacokinetic parameters of indisulam for the patients included in this study were obtained from the population parameters of the previously published pharmacokinetic and pharmacogenetic model and the data from the current study, using the POSTHOC option of NONMEM [21].

Pharmacokinetic model of carboplatin The pharmacokinetic results of ultrafiltrable carboplatin were fitted to an open two-compartment model with first-order elimination (Figure 1) [22–24]. The clearance of carboplatin ($\text{CL}_{\text{carboplatin},i}$, Equation 2) was estimated as a linear function of the creatinine clearance, as predicted by the Cockcroft–Gault formula ($\text{CL}_{\text{creatinine CG},i}$, Equation 1). In Equation 2,

Figure 1

Structural models to describe indisulam pharmacokinetics, carboplatin pharmacokinetics and haematological toxicity. INDISULAM: $B_{\max}$, maximal specific binding capacity; K_D , equilibrium dissociation constant; N , non-specific binding constant; Q , intercompartmental clearance; CL , clearance; $V_{\max}$, Michaelis–Menten maximal elimination rate; K_m , Michaelis–Menten constant. CARBOPLATIN: CL , clearance; V_1 , central volume of distribution; V_2 , peripheral volume of distribution; Q , intercompartmental clearance. HAEMATOLOGICAL TOXICITY: k_{prol} , proliferation rate constant of progenitor blood cells; k_{tr} , transition rate constant

$CL_{\text{creatinine CG},i} \times \theta(1)$ represents the typical renal clearance and $\theta(2)$ corresponds to the typical nonrenal clearance of carboplatin, which has previously been estimated at 1.5 l h^{-1} by Calvert *et al.* [16].

$$CL_{\text{creatinine CG},i} (\text{l h}^{-1}) = \frac{[140 - \text{age}] * \text{weight (kg)} * 0.85 \text{ (for female)} * 0.074}{\text{serum creatinine } (\mu\text{M})} \quad (1)$$

$$CL_{\text{carboplatin},i} (\text{l h}^{-1}) = [CL_{\text{creatinine CG},i} \times \theta(1) + \theta(2)] \times \exp(\eta_i) \quad (2)$$

Pharmacokinetic–pharmacodynamic model for myelosuppression The time courses of neutropenia and thrombocytopenia were described by a semiphysiological model,

which has been introduced by Friberg *et al.* and has previously been applied to describe the haematological effect of indisulam by us [25, 26]. This model comprised a progenitor compartment for proliferating blood cells, linked to a series of three compartments representing the maturation chain in the bone marrow and leading to the central circulation compartment (Figure 1).

Two system related parameters were estimated: mean transit time ($MTT_{\text{blood cells}}$) and a feedback parameter gamma ($\gamma_{\text{blood cells}}$). The $MTT_{\text{blood cells}}$ was the average time between cell proliferation and completion of maturation and was related to the first-order transition rate constant $k_{\text{tr blood cells}} (= 4/MTT_{\text{blood cells}})$. The feedback parameter represented the induction of stem cell proliferation by

endogenous growth factors and/or cytokines [27]. Myelosuppressive effects of both drugs were estimated. The proliferation rate was reduced by the effect of indisulam ($E_{\text{indisulam}}$) and/or carboplatin ($E_{\text{carboplatin}}$) according to linear functions [$k_{\text{prol}} = k_{\text{tr blood cells}} \cdot (1 - E_{\text{indisulam}} - E_{\text{carboplatin}})$]. The myelosuppressive effects of indisulam and carboplatin were supposed to be proportional to the plasma concentrations of indisulam ($E_{\text{indisulam}} = \text{slope}_{\text{indisulam}} \cdot C_{\text{indisulam}}$) and unbound carboplatin ($E_{\text{carboplatin}} = \text{slope}_{\text{carboplatin}} \cdot C_{\text{carboplatin}}$).

Exposure to indisulam might enhance the efficacy and/or toxicity of carboplatin (see Introduction and Discussion). An interaction term was estimated to quantify the potential synergism of indisulam and carboplatin in myelosuppression (Equation 3) (additive effect: $\text{slope}_{\text{inter}} = 0$; antagonism: $\text{slope}_{\text{inter}} < 0$; synergism: $\text{slope}_{\text{inter}} > 0$).

$$k_{\text{prol}} = k_{\text{tr blood cells}} \cdot (1 - E_{\text{indisulam}} - E_{\text{carboplatin}} - \text{slope}_{\text{inter}} \cdot C_{\text{carboplatin}} \cdot C_{\text{indisulam}}) \quad (3)$$

Transfusion of thrombocytes was modelled as a bolus dose into the last compartment of the semiphysiological model, representing the central circulation. The volume of distribution corresponded to the blood volume (BL). This is known to be related to the BSA (m^2) and was calculated using Equation 4a for men and Equation 4b for women [28].

$$\text{BL}_{\text{male}} = (3.29 \cdot \text{BSA} - 1.229) \quad (4a)$$

$$\text{BL}_{\text{female}} = (3.47 \cdot \text{BSA} - 1.954) \quad (4b)$$

Pharmacodynamic parameters were estimated conditionally on the population pharmacokinetic parameters of indisulam and carboplatin, which were fixed in this stage of model development. All data (concentrations of indisulam and ultrafiltrable carboplatin, neutrophil and thrombocyte counts) were used in the PK–PD analysis, in order to obtain MAP Bayesian estimates of the individual pharmacokinetic parameters of indisulam and carboplatin and to estimate the pharmacodynamic population parameters of neutropenia and thrombocytopenia. This method has been described in detail by Zhang *et al.* [29].

Model evaluation

The pharmacodynamic model of myelosuppression was evaluated by a case-deletion diagnostic procedure and a numerical predictive check. The case-deletion diagnostic procedure was performed to assess bias of parameter estimates [30]. Sixteen consecutive analyses were performed using data from 15 out of 16 patients. If the parameter estimates of a case-deletion diagnostic procedure were similar to the estimates resulting from the full dataset, the parameter estimates were considered not to be highly dependent on single individuals. For the numerical predictive check, 1000 datasets were simulated from the parameter estimates of the final model and for each observation

the 90% prediction interval was defined. The model was considered unbiased if approximately 10% of the neutrophil and thrombocyte counts (randomly distributed over the time and observation range) were below or above the prediction interval.

Simulation study

A simulation study was performed to evaluate the tolerability of various dose levels of the combination indisulam–carboplatin in 3-weekly and 4-weekly treatment schedules. Using the final pharmacokinetic and pharmacodynamic model of neutropenia and thrombocytopenia, cohorts of 10 000 patients were simulated with the SIMULATION option of NONMEM to determine the risk of treatment delay and dose-limiting myelosuppression using the parameter estimates from the model developed on data from the clinical study.

Relevant patient characteristics (Table 1) were simulated from geometric means and variances that were derived from a larger population of 412 patients (including the current 16 patients) that have been treated with indisulam [31]. These geometric means and variances were determined for men and women independently (e.g. $\text{WT}_{\text{males}} 77.1 \text{ kg} \pm 21\%$ and $\text{WT}_{\text{males}} 63.7 \text{ kg} \pm 15\%$) and were subsequently used to simulate patient cohorts consisting of equal proportions of men and women. Furthermore, a CYP2C genotype was randomly assigned to all patients. The allele frequencies of the relevant CYP2C9*3 and CYP2C19*2 polymorphisms were 8.4 and 14.7%, respectively. These relative frequencies are representative of a Caucasian patient population [32]. The reported frequency of CYP2C19*3 was low (0.04%). This polymorphism was therefore not considered relevant for the simulation study.

The carboplatin dose (mg) for each patient was calculated from the target AUC ($\text{mg min}^{-1} \text{ mL}^{-1}$) and the simulated patient characteristics, using Equations 1 and 5. A random effect was added to determine the individual carboplatin clearance using Equation 2. Consequently, the

Table 1

Characteristics of the patients included in the dose escalation study and in the pharmacokinetic–pharmacodynamic analysis

		Median	Range
Age	(years)	63	19–81
Body surface area	(m^2)	1.73	1.36–2.22
Weight	(kg)	66	43–116
Serum creatinine	($\mu\text{mol L}^{-1}$)	74	33–132
n			
Race	Caucasian	16	
Gender	Male	11	
	Female	5	

carboplatin AUC in the simulated patients was randomly distributed around the target AUC.

$$\text{Dose}_{\text{carboplatin}} = \text{target AUC} \times [\text{CL}_{\text{creatinine CG},1} \times \theta(1) + \theta(2)] \times 1000/60 \quad (5)$$

Treatment delay was indicated for patients with grade ≥ 2 neutropenia and/or thrombocytopenia (absolute neutrophil count $< 1.5 \times 10^9 \text{ l}^{-1}$; thrombocyte count $< 100 \times 10^9 \text{ l}^{-1}$) at the planned time of dosing. DLT was defined as grade 4 neutropenia (absolute neutrophil count $< 0.5 \times 10^9 \text{ l}^{-1}$) during > 7 days or grade 4 thrombocytopenia (thrombocyte count $< 10 \times 10^9 \text{ l}^{-1}$). The proportion of patients with dose-limiting myelosuppression and/or treatment delay in each simulated cohort was equal to the risk of an individual patient. Various dose combinations of indisulam (350, 500, 600 and 700 mg m^{-2}) and carboplatin (AUC 4, AUC 5 and $\text{AUC } 6 \text{ mg min}^{-1} \text{ ml}^{-1}$) were planned to be evaluated. In accordance with the common definition of a non-tolerated dose in dose escalation studies, a dosing regimen was considered nontolerated if two or more out of six patients ($\geq 33\%$) had an overall risk of dose-limiting myelosuppression and/or treatment delay. If the risk of DLT and/or treatment delay was $< 33\%$, the tolerability was considered acceptable.

Results

Patients and data

Four dose levels of the combination indisulam (mg m^{-2})/carboplatin ($\text{mg min}^{-1} \text{ ml}^{-1}$) were evaluated in the clinical study: 350/6, 500/6, 600/6, 600/5. In total, 16 patients were treated with the combination. Patient characteristics are listed in Table 1. In total, 53 cycles were administered (median 2, range 1–7). Of 37 doses that were administered after the first treatment cycle, 30 were delayed due to unresolved thrombocytopenia and/or neutropenia. During the course of the study, 11 patients (69%) had grade 3 or 4 thrombocytopenia according to the Common Toxicity Criteria (CTC) and 10 patients (62.5%) had CTC grade 3 or 4 neutropenia. During follow-up, no patients were treated with G-CSF to support recovery from severe neutropenia. Thrombocyte concentrates were administered to four different patients in a total of six treatment cycles. Pharmacokinetic and haematology data were available for all patients.

Population pharmacokinetic and pharmacodynamic model development

Pharmacokinetic model of indisulam The time profile of the indisulam plasma concentrations ($n = 218$) was nonlinear (Figure 2a) and could be described by the semiphysiological pharmacokinetic and pharmacogenetic model (Figure 2b). Variability of exposure to indisulam was

large. The AUC varied more than eightfold from 400 to $3300 \text{ mg h}^{-1} \text{ l}^{-1}$, which could be partly explained by differences in dose levels ($350\text{--}600 \text{ mg m}^{-2}$) in combination with the nonlinear pharmacokinetics, and partly by genetic variation. In this study, nine patients had a CYP2C genotype that was related to a reduced elimination of indisulam (CYP2C9*3, $n = 6$; CYP2C19*2, $n = 3$).

Pharmacokinetic model of carboplatin Concentrations of carboplatin in plasma ultrafiltrate ($n = 111$) could be well described by an open linear two-compartment model (Figure 2c,d). $\theta(2)$ (Equation 2) was not significantly different from 1.5 l h^{-1} (Calvert *et al.* [16]) and was subsequently fixed to this value. The population parameter estimates of the final model are listed in Table 2. The AUC of carboplatin was targeted at 5 or $6 \text{ mg min}^{-1} \text{ ml}^{-1}$ for all patients, but actual exposure varied between 5 and $10 \text{ mg min}^{-1} \text{ ml}^{-1}$. This was reflected in the estimate of 0.76 for $\theta(1)$, which indicates that creatinine clearance as predicted by the Cockcroft–Gault formula was higher than renal clearance of carboplatin in this study.

Pharmacokinetic–pharmacodynamic model of myelosuppression In total, 247 measurements of the absolute neutrophil count and 241 thrombocyte counts were available. These pharmacodynamic data could be adequately described by the PK-PD models of neutropenia and thrombocytopenia (Figure 3). In Table 3, the pharmacodynamic parameters are listed. All system-related parameters (MTT, gamma) could be precisely estimated. The data did not contain sufficient information to estimate the slope for indisulam and for carboplatin independently. The slopes of indisulam were set to the values that were previously determined based on indisulam monotherapy [26]. A random effect of the inhibition of the proliferation rate was estimated for each type of blood cell (Table 3). In addition, a correlation coefficient was estimated on this random effect to capture co-variance between the severity of neutropenia and thrombocytopenia between patients. This correlation coefficient was estimated to be 0.61.

The total number of thrombocytes in the concentrates administered to patients was not recorded and was therefore estimated in the PK–PD analysis (4.01×10^{11}). This estimate was in accordance with the content of one unit of the thrombocyte concentrate product that was used for thrombocyte transfusions ($2\text{--}4 \times 10^{11} \text{ cells}$).

The estimates of slope indicated that exposure to indisulam had a large impact on the risk of neutropenia ($\text{slope}_{\text{neutrophils}} 0.054 \text{ l } \mu\text{mol}^{-1} > \text{slope}_{\text{thrombocytes}} 0.018 \text{ l } \mu\text{mol}^{-1}$), whereas exposure to carboplatin highly influenced the risk of thrombocytopenia ($\text{slope}_{\text{neutrophils}} 0.216 \text{ l } \mu\text{mol}^{-1} > \text{slope}_{\text{thrombocytes}} 0.300 \text{ l } \mu\text{mol}^{-1}$). Synergism of indisulam and carboplatin in myelosuppression could not be demonstrated. For both the neutropenic and thrombocytopenic effects, the interaction terms were not significantly different from 0.

Figure 2

Pharmacokinetic profiles (a,c) and goodness of fit plots (b,d). Observed concentrations (○) and individual predicted profiles (—) show the nonlinear pharmacokinetic profile of indisulam (a) and the biphasic elimination of unbound carboplatin (c). Model-predicted concentrations are plotted vs. observed concentrations to visualize the goodness of fit for the pharmacokinetic models of indisulam (b) and carboplatin (d)

Model evaluation

The results of the case-deletion diagnostic procedure are listed in Table 3. Parameter estimates that were obtained with data from 15 out of 16 patients corresponded well to the estimates that were obtained with all data. Only the dose of thrombocytes that was administered during a transfusion was highly dependent on single patients,

because only four individuals received one or more thrombocyte transfusions.

A predictive check was performed for all neutrophil and thrombocyte counts. Of 241 observed neutrophil counts, nine (3.7%) were below the prediction interval. Of 247 thrombocyte counts, 18 (7.3%) were below the prediction interval. These numbers were close to the ideal 5% of all observations. However, only one observation was above

Table 2
Population pharmacokinetic parameter estimates of ultrafiltrable carboplatin

		Estimate	(RSE)	IIV	(RSE)
Clearance (CL)	(l h ⁻¹)	CL _{creatinine} CG · 0.76*+1.5**	(0.05)*	13%	(0.27)
Volume of central compartment	(l)	15.5	(0.19)	54%	(1.46)
Intercompartmental clearance	(l h ⁻¹)	3.46	(0.18)	46%	(0.39)
Volume of peripheral compartment	(l)	9.86	(0.11)	31%	(0.41)
Residual error	(%)	8.2	(0.11)		

*θ(2) in Equation 2 was estimated at 0.76 (RSE = 0.05). **The non-renal clearance of carboplatin was fixed at 1.5 l h⁻¹. IIV, unexplained interindividual variability; RSE, relative standard error.

the prediction interval. This may indicate model misspecification, but it did not affect the results of this study, because the simulated risks of DLT and treatment delay were determined based on the proportion of patients with low nadir blood cell counts.

Simulation study

Table 4 and Figure 4 show the simulated risks of treatment delay and DLT for a range of dose combinations of indisulam and carboplatin. For all 3-weekly regimens, the risk of treatment delay was higher than the target risk of 33%. Even for the combination of the two lowest dose levels (350 mg m⁻² indisulam and 4 mg min⁻¹ ml⁻¹), the risk of dose delay was estimated to be 34%. The risk further increased with increasing doses of both indisulam and carboplatin. In Table 4, the risks of thrombocytopenia and neutropenia are listed separately. The predicted risks were lower for thrombocytopenia than for neutropenia, as opposed to the results of the clinical study, where the incidences of thrombocytopenia and neutropenia were similar (see Discussion). The 4-weekly regimens were better tolerated. The risk of treatment delay in a 4-weekly regimen was predicted to be 9–24%.

The risk of DLT and/or treatment delay was considered to select a regimen with acceptable tolerability (risk < 33%) and optimal dose intensity. For the combination of 500 mg m⁻² indisulam and 6 mg min⁻¹ ml⁻¹ carboplatin in a 4-weekly regimen, the risk of DLT and/or treatment delay was 32%. This dosing regimen seems to be acceptable regarding tolerability and optimal regarding dose intensity.

Discussion

In the Phase I dose escalation study of the investigational cell-cycle inhibitor indisulam in combination with carboplatin, only 16 patients were treated at various dose levels. Due to wide variability between individuals, the study outcome might be highly influenced by the random patient selection. In the clinical study, three out of four patients had a DLT at the 600 mg m⁻² indisulam/6 mg min⁻¹ ml⁻¹ carboplatin dose level, which was there-

fore defined as the non-tolerated dose level. No DLTs were observed in three other patients who were treated with a lower indisulam dose of 500 mg m⁻² in combination with the same dose of carboplatin to achieve an AUC of 6 mg min⁻¹ ml⁻¹, but two out of two patients who received a second treatment cycle at this dose level required a dose delay of 1 week. The large difference in the incidence of DLTs between these two dose levels was probably partly related to patient selection, and it remained unclear to what extent the extra 100 mg m⁻² of indisulam contributed to the observed toxicities. This could, however, be quantified by the presented population PK–PD analysis. In this integrated analysis, all pharmacokinetic and pharmacodynamic data were combined to evaluate the safety of various dose levels in a patient population, taking into account the variability between patients.

The exposure to carboplatin in this study was higher than anticipated. All patients received an individual dose of carboplatin that was determined using a modified Calvert formula where the creatinine clearance, as predicted with the Cockcroft–Gault equation, was used as an estimate of the glomerular filtration rate [16]. The presented population pharmacokinetic analysis showed that the observed carboplatin clearance in this study was systematically lower than the predictions from the modified Calvert and Cockcroft–Gault formulae. This may be explained by different methods of determination of creatinine levels that were used in this study and by Cockcroft and Gault.

Serum creatinine levels were previously measured with the alkaline picrate method of Jaffe. Serum creatinine levels are overestimated by this method as a result of the interference of noncreatinine chromogens. In the current study, serum creatinine levels were measured by more accurate enzymatic methods. Although serum creatinine is often assumed to be eliminated only by passive renal elimination, active tubular secretion accounts for about 20% of creatinine clearance [33]. The tubular secretion used to be counterbalanced by the overestimation of serum creatinine levels. However, in this study the lack of compensation for the tubular secretion of creatinine may have caused an overestimation of the glomerular filtration rate and consequently of carboplatin clearance.

Figure 3

Goodness of fit plots for the pharmacodynamic model: weighted residuals vs. time (a,c) and individual predictions vs. observed neutrophil counts and thrombocyte counts (B,D)

The overestimation of carboplatin clearance by the modified Calvert and Cockcroft–Gault formulae resulted in higher exposure to carboplatin compared with the target exposure in the clinical study. In the simulation study, however, the typical AUC corresponded to the target AUC of carboplatin. This may explain why the incidence of thrombocytopenia, the major toxicity of carboplatin, was similar to the incidence of neutropenia in the clinical study,

whereas the risk of thrombocytopenia was lower than the risk of neutropenia in the simulation study.

The myelosuppressive effect of indisulam was supposed to be proportional to the plasma concentration of indisulam. In a previous study, our group demonstrated that a linear model was preferred over an $E_{\max}$ model to describe the concentration–effect relationship for indisulam [26]. For carboplatin, it has not been demonstrated

Table 3

Population pharmacodynamic parameter estimates for the semiphysiological model of myelosuppression after treatment with indisulam and carboplatin

	Estimate	(RSE)	(Range)*	IIV	(RSE)	(Range)*
Absolute neutrophil count						
MTT (h)	178	(0.04)	(175–184)	17%	(0.23)	(16–19%)
Gamma	0.147	(0.08)	(0.143–0.155)	–	–	–
Slope indisulam ($\text{l } \mu\text{mol}^{-1}$)	0.054	(fixed)	–	61%†	(0.64)	(41–67%)
Slope carboplatin ($\text{l } \mu\text{mol}^{-1}$)	0.216	(0.08)	(0.208–0.228)	–	–	–
Residual error (%)	48	(0.12)	(42–50)	–	–	–
Thrombocyte count						
Dose thrombocyte transfusion	401·10 ⁹	(0.54)	(136–719)	–	–	–
MTT (h)	142	(0.06)	(125–148)	16%	(0.53)	(14–27%)
Gamma	0.176	(0.08)	(0.155–0.182)	–	–	–
Slope indisulam ($\text{l } \mu\text{mol}^{-1}$)	0.018	(fixed)	–	26%†	(0.76)	(19–33%)
Slope carboplatin ($\text{l } \mu\text{mol}^{-1}$)	0.300	(0.09)	(0.258–0.320)	–	–	–
Residual error (%)	56	(0.13)	(48–59)	–	–	–

*Range of estimates from case-deletion diagnostic procedure. †The correlation coefficient between the inhibitory effects of the combination for neutrophils and thrombocytes was estimated at 0.61. RSE, relative standard error; IIV, interindividual variability.

that a linear model was preferred over an E_{max} model. Data from the current study did not contain sufficient information to evaluate E_{max} models for carboplatin-induced neutropenia and thrombocytopenia. However, Friberg *et al.* have demonstrated that linear models could adequately describe the concentration–effect relationship of various myelosuppressive anticancer drugs [25].

The inhibitory effects of indisulam and carboplatin on the proliferation rate of neutrophils and thrombocytes could not be estimated independently in the current analysis. The effects of indisulam were therefore set to the previously determined values for monotherapy [26]. This was considered feasible, because there is no indication that co-treatment with carboplatin would affect the cytotoxic effect of indisulam, which was administered 1 day prior to carboplatin. Conversely, indisulam might enhance the myelotoxicity of carboplatin. Preclinical studies demonstrated a synergistic effect of the combination when indisulam was administered prior to carboplatin. This synergism might not only play a role in the antitumour effect of the combination, but also increase the toxicity. Although synergistic toxicity could not be demonstrated in the current analysis, the presented pharmacodynamic model of myelosuppression should only be used to describe and predict the time course of thrombocytes and/or neutrophils after combination treatment with indisulam and carboplatin as opposed to single-agent therapy.

The MTT in the semiphysiological model of myelosuppression is a system-related parameter, and its value was shown to be consistent across drugs for neutrophils (geometric mean 116 h, geometric coefficient of variance 18% for five anticancer drugs) [25]. For indisulam, the MTT of neutrophils was estimated to be 156 h and the MTT of thrombocytes was estimated to be 103 h in patients who were treated with indisulam monotherapy. In the current

analysis of indisulam in combination with carboplatin, the estimates of the MTT for both neutrophils and thrombocytes were larger than expected. This may be explained by a difference between the pharmacokinetic profile of unbound carboplatin in plasma and the pharmacokinetic profile of activated intracellular carboplatin. The terminal half-life of unbound carboplatin in plasma was estimated to be 4.4 h in this study, for a typical male patient (age 60 years, weight 75 kg, serum creatinine $90 \mu\text{mol l}^{-1}$), but the half-life of an activated intracellular metabolite of carboplatin (resulting from an activating reaction with L-methionine) might be as long as 28 h [34]. The sustained intracellular activity of carboplatin might be reflected by large estimates for MTT in this study. Alternatively, indisulam and/or carboplatin may affect cell differentiation of blood cells, in addition to the known inhibition of the proliferation rate of progenitor cells.

Indisulam was evaluated in combination with carboplatin in a Phase I dose escalation study to assess the tolerability of the combination and to select a safe dose for future Phase II development. It was concluded that the combination was not well tolerated in a 3-weekly treatment regimen in the clinical study, although only a very limited number of patients was treated. The current simulation study, which took into account the data from the clinical study and extensive PK–PD knowledge from indisulam monotherapy, has demonstrated that a 4-weekly regimen is better tolerated than a 3-weekly regimen. The risk of dose-limiting myelosuppression and/or a treatment delay to recover from myelosuppression was estimated to be 32% for a 4-weekly regimen of 500 mg m^{-2} indisulam and $6 \text{ mg min}^{-1} \text{ ml}^{-1}$ carboplatin. This regimen was considered safe and feasible for Phase II evaluation in patients with locally advanced or metastatic NSCLC.

Table 4

Results of the simulation study

Dose indisulam (mg m ⁻²)	Target dose carboplatin (mg min ⁻¹ ml)	Risk of dose-limiting myelosuppression during first cycle (DLT)			Risk of delay at cycle 2 due to myelosuppression 3-weekly regimen			Risk of delay at cycle 2 due to myelosuppression 4-weekly regimen			Delay and/or DLT 4-weekly regimen	
		T	N	T/N	T	N	T/N	T	N	T/N	T/N	
350	4	0.5%	5.8%	6.1%	15%	22%	34%	2.9%	6.5%	9.2%	13%	
500	4	1.1%	11%	12%	16%	32%	41%	3.0%	10%	13%	20%	
600	4	1.4%	15%	16%	17%	37%	46%	2.7%	13%	15%	24%	
700	4	1.8%	20%	20%	18%	43%	52%	3.4%	16%	18%	29%	
350	5	2.0%	9.2%	10%	19%	27%	40%	3.2%	8.8%	12%	18%	
500	5	2.9%	15%	16%	21%	38%	49%	3.0%	13%	16%	25%	
600	5	3.9%	21%	22%	24%	43%	55%	3.5%	16%	19%	31%	
700	5	4.8%	24%	26%	25%	49%	60%	3.5%	17%	20%	35%	
350	6	5.6%	13%	17%	26%	34%	50%	4.0%	11%	15%	25%	
500	6	6.4%	19%	23%	27%	43%	57%	3.6%	15%	18%	32%	
600	6	8.2%	25%	29%	29%	50%	62%	3.6%	18%	21%	38%	
700	6	9.3%	29%	33%	30%	53%	65%	3.7%	21%	24%	41%	

T/N, thrombocytopenia and/or neutropenia; T, thrombocytopenia; N, neutropenia.

Figure 4

Graphical representation of the results of the simulation study. (a) The predicted risk of a treatment delay in 3-weekly (■) and 4-weekly treatment regimens (■) of various dose combinations of indisulam and carboplatin. In all 3-weekly regimens, the risk was unacceptably high. (b) The predicted overall risk of dose-limiting myelosuppression and/or treatment delay after 4 weeks for the various dose combinations. The risk was estimated at 32% for the combination 500 mg m⁻² indisulam and 6 mg min⁻¹ ml⁻¹ carboplatin

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