Population pharmacokinetics and concentration–effect relationships of capecitabine metabolites in colorectal cancer patients

Ronald Gieschke, Hans-Ulrich Burger, Bruno Reigner,¹ Karen S. Blesch² & Jean-Louis Steimer

Biostatistics and ¹Clinical Pharmacology, Pharma Development, F. Hoffmann-La Roche Ltd, Basel, Switzerland, and ²Biometrics, Pharma Development, F. Hoffmann-La Roche, Inc., Nutley, NJ, USA

Aims To assess the relationship between systemic exposure to capecitabine metabolites and parameters of efficacy and safety in patients with advanced or metastatic colorectal cancer from two phase III studies.

Methods Concentration–effect analyses were based on data from 481 patients (248 males, 193 females; age range 27–86 years) in two phase III studies. Plasma concentration–time data for 5′-deoxy-5-fluorouridine (5′-DFUR), 5-fluorouracil (5-FU) and α -fluoro- β -alanine (FBAL) were obtained from sparse blood samples collected within the time windows 0.5–1.5 h, 1.5–3.0 h, and 3.0–5.0 h after capecitabine administration (1250 mg m⁻²) on the first day of cycles 2 (day 22) and 4 (day 64), respectively. Systemic exposure based on plasma concentrations of capecitabine and its metabolites was determined using individual parameter estimates derived from a population pharmacokinetic model constructed for this purpose in NONMEM. Logistic regression analysis was conducted for selected safety parameters (all treatment-related grade 3–4 adverse events, treatment-related grade 3–4 diarrhoea, grade 3 hand–foot syndrome (HFS) and grade 3–4 hyperbilirubinaemia) and for tumour response. Cox regression analysis was used for the analysis of time-to-event data (time to disease progression and duration of survival).

Results Statistically significant relationships between covariates and PK parameters were found as follows. A doubling of alkaline phosphatase activity was associated with a 11% decrease in 5-FU clearance and a 12% increase in its AUC. A 50% decrease in creatinine clearance was associated with a 35% decrease in FBAL clearance, a 53% increase in its AUC, a 24% decrease in its volume of distribution, and a 41% increase in its $C_{\max}$. A 30% increase in body surface was associated with a 24% increase in the volume of distribution of FBAL and a 19% decrease in its $C_{\max}$. There was a broad overlap in systemic drug exposure between patients regardless of the occurrence of treatment-related grade 3–4 adverse events or response to treatment, leading to weak relationships between systemic exposure to capecitabine metabolites and the safety and efficacy parameters. Of 42 concentration–effect relationships investigated, only five achieved statistical significance. Thus, we obtained a positive association between the AUC of FBAL and grade 3–4 diarrhoea ($P = 0.035$), a positive association between the AUC of 5-FU and grade 3–4 hyperbilirubinaemia ($P = 0.025$), a negative association between the $C_{\max}$ of FBAL and grade 3–4 hyperbilirubinaemia ($P = 0.014$), a negative association between the AUC of 5-FU (in plasma) and time to disease progression (hazard ratio (HR) = 1.626, $P = 0.0056$), and a positive association between the $C_{\max}$ of 5′-DFUR and survival (HR = 0.938, $P = 0.0048$). Additionally, there were inconsistencies when concentration–effect relationships were compared across the two studies.

Conclusions Systemic exposure to capecitabine and its metabolites in plasma is poorly predictive of safety and efficacy. The present results have no clinical impli-

Correspondence: Ronald Gieschke MD, Clinical Pharmacology, Pharma Development, F. Hoffmann-La Roche Ltd, PDMP, 15/I.030, CH-4070 Basel, Switzerland. Tel.: + 41 61 688 4820; E-mail: ronald.gieschke@roche.com

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cations for the use of capecitabine and argue against the value of therapeutic drug monitoring for dosage adjustment.

Keywords: 5-FU, capecitabine, colorectal cancer, pharmacokinetic and pharmacodynamic modelling

Introduction

Investigating the relationships between systemic exposure to antineoplastics and their safety and efficacy in cancer treatment is an important aspect of the development of cancer chemotherapeutic agents [1]. The pharmacokinetics and pharmacodynamics of 5-FU have been investigated extensively in the treatment of colorectal and other cancers, including attempts to predict safety and efficacy [2–6]. More recently, complex modelling techniques have been developed to further elucidate concentration–effect relationships in cancer chemotherapy [7–10].

Capecitabine is an orally administered tumour-selective fluoropyrimidine that is metabolized primarily in the liver by the 60-kDa carboxylesterase to 5'-deoxy-5-fluorocytidine (5'-DFCR). The latter is then converted to 5'-deoxy-5-fluorouridine (5'-DFUR) by cytidine deaminase, which is principally located in the liver and tumour tissues. 5'-DFUR is converted to the active moiety, 5-fluorouracil (5-FU) by thymidine phosphorylase (TP), which is present at considerably higher concentrations in tumour tissues than in normal tissues [11]. 5-FU is either metabolized through multiple steps to an active phosphate analogue or is catabolized to 5,6-dihydro-5-fluorouracil (FUH₂), α -fluoro- β -ureido propionic acid (FUPA) and ultimately to α -fluoro- β -alanine (FBAL) [12], which is excreted in urine.

The relationship between exposure to capecitabine metabolites and the occurrence of adverse effects has been reported in phase I studies [13]. $C_{\max}$ and AUC for 5'-DFUR and FBAL were found to be predictive of dose-limiting toxicities (DLT), whereas systemic exposure to 5-FU was poorly predictive. The objective of the present analysis was to investigate further the relationships between systemic exposure (AUC and $C_{\max}$) to the capecitabine metabolites 5'-DFUR, 5-FU, and FBAL and safety and efficacy outcomes in colorectal cancer patients. Values for AUC and $C_{\max}$ were derived from a population pharmacokinetic (PK) model constructed for this purpose.

Methods

Patients

A total of 481 patients with advanced or metastatic colorectal cancer from two phase III studies [14, 15], and

24 patients with various advanced solid tumours from a bioequivalence study [16] were included in the population PK model building. Concentration–effect analyses were then conducted utilizing the PK model with the 481 patients from the phase III studies. Baseline disease and demographic characteristics for the patients included in these analyses are presented in Table 1. Only those variables considered as potential covariates for developing the population pharmacokinetic model are shown.

Study design

In the bioequivalence study, a single dose of 2000 mg capecitabine was administered orally within 5 min of a standard breakfast. Plasma concentration–time data for 5'-DFUR, 5-FU and FBAL were obtained just prior to dosing, and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, and 12 h after drug intake. For the two open-label phase III studies, patients were randomized to receive either 1250 mg m⁻² bid⁻¹ of capecitabine intermittently (2 weeks on and 1 week off) or 5-FU in combination with leucovorin over at least 6 weeks. Only patients randomized to receive capecitabine were included in this analysis. Plasma concentration–time data for 5'-DFUR, 5-FU and FBAL were obtained from sparse blood samples collected within the time windows 0.5–1.5 h, 1.5–3.0 h, and 3.0–5.0 h after drug intake on the first day of cycles 2 (day 22) and 4 (day 64), respectively.

The clinical studies were approved by local ethics committees and were conducted according to the guidelines of the Declaration of Helsinki. All subjects gave their written informed consent.

Analytical procedure

The analytical procedure has been described in detail by Cassidy *et al.* [16]. Briefly, blood samples were collected and plasma harvested. Plasma samples were analysed by liquid chromatography (LC)–tandem mass spectrometry (MS/MS). The assay was validated and the limit of quantification for 5'-DFUR, 5-FU, and FBAL was 0.05 $\mu\text{g ml}^{-1}$ (0.2 $\mu\text{mol l}^{-1}$), 0.002 $\mu\text{g ml}^{-1}$ (0.015 $\mu\text{mol l}^{-1}$), and 0.02 $\mu\text{g ml}^{-1}$ (0.19 $\mu\text{mol l}^{-1}$), respectively. The overall between-day variabilities of the quality control (QC) samples were <6% for 5'-DFUR, <8% for 5-FU, and <11% for FBAL. The QC deviations from nominal con-

Table 1 Baseline demographic and disease characteristics of patients included in the analysis.

Covariate	Phase III study (14) n, mean $\pm$ SD, min–max	Phase III study (15) n, mean $\pm$ SD, min–max	Bioequiv. study (16) n, mean $\pm$ SD, min–max
Age (years)	248, 61 $\pm$ 10, 29–80	233, 62 $\pm$ 11, 27–86	24, 63 $\pm$ 10, 41–80
Weight (kg)	248, 70 $\pm$ 13, 42–120	232, 77 $\pm$ 20, 35.8–208.7	24, 73 $\pm$ 17, 41.5–103
BSA (m ²)*	248, 1.8 $\pm$ 0.2, 1.37–2.36	232, 1.9 $\pm$ 0.2, 1.19–3.06	24, 1.8 $\pm$ 0.2, 1.37–2.34
Creatinine clearance (ml min ⁻¹)†	247, 77 $\pm$ 24, 31–155	230, 89 $\pm$ 32, 26–261	24, 82 $\pm$ 22, 42–129
Gender	148 M/100 F	140 M/93 F	15 M/9 F
Race (Caucasian/black/other)	233/0/15	198/22/13	24/0/0
Karnofsky scale (70/80/90–95/100)	22/48/85/90	29/39/92/71	1/5/11/7
Liver metastasis	183 yes/65 no	177 yes/56 no	4 yes/20 no
Predominant site of disease: liver	171 yes/77 no	168 yes/65 no	–
Albumin (g l ⁻¹)	219, 39 $\pm$ 6, 4–53	224, 39 $\pm$ 5, 21–50	–
ASAT (U l ⁻¹)	238, 28 $\pm$ 18, 6–150	215, 34 $\pm$ 23, 9–152	22, 21 $\pm$ 12, 9–52
ALAT (U/l)	244, 26 $\pm$ 21, 3–201	218, 26 $\pm$ 19, 2–144	21, 20 $\pm$ 11, 6–46
ALP (U/l)	243, 245 $\pm$ 201, 42–1346	232, 170 $\pm$ 138, 45–962	24, 142 $\pm$ 100, 44–494
Bilirubin, total (μmol/l)	241, 9.2 $\pm$ 4.1, 1.7–29.0	229, 10.2 $\pm$ 4.3, 1.7–26.7	24, 6.5 $\pm$ 2.9, 2.3–12.4

*Body surface area (BSA) values were taken from a nomogram implementing the formula from DuBois and DuBois [33]. $BSA = 71.84 \times BW^{0.425} \times Height^{0.725}$, where BW stands for bodyweight in kg, Height for height in cm, and BSA for body surface area in cm². †Creatinine clearance values were calculated according to the formula of Cockcroft and Gault [34]: $CLCR = BW \times (140 - AGE)/SCREA/72$ for males and $CLCR = 0.85 \times BW \times (140 - AGE)/SCREA/72$ for females, where BW stands for body weight in kg, AGE for age in years, SCREA for serum creatinine in mg dl⁻¹, and CLCR for creatinine clearance in ml min⁻¹.

centrations were within 10% for 5'-DFUR, within 13% for 5-FU, and within 4% for FBAL. The overall between-day variabilities of the calibration standards were <6% for 5'-DFUR, <7% for 5-FU and <10% for FBAL. The coefficients of determination for the calibration curves for 5'-DFUR, 5-FU and FBAL ranged from 0.9838 to 0.9995.

Population pharmacokinetic model development

The population PK model was originally developed utilizing data from a phase II study of breast cancer and extensively sampled PK data from the bioequivalence study [16]. The methodology has been reported in detail elsewhere [17]. In addition to the original modelling work, validation of the model has been attempted using data-splitting and predicting concentrations and parameters [18] as follows. The combined data from the two phase III studies were randomly split into an 'index' data set (2/3 of all data) and a 'validation' (1/3 of all data) data set. The appropriateness of the split was confirmed using the Wilcoxon test (for continuous covariates) and the χ^2 test (for categorical covariates) at a significance level of $\alpha = 0.05$. Next, the index data set was used to develop a population (covariate) model, based on the structural PK model from the bioequivalence study (Figure 1). This population model was then used to predict drug concentrations and PK parameters for the validation data set, on the basis of covariates in the validation data set. The predicted concentrations for the

validation data set were then compared with the actual measured plasma concentrations in the validation data set, and respective means and mean squared errors were calculated for all measurements [19]. The predicted PK parameters were compared with 'reference' PK parameters that had been estimated for the validation data set using observed plasma concentrations and the PK model without any covariates [20].

As described previously [17], the following error models were applied. Unexplained intersubject variability (ISV) in PK model parameters was estimated using the following model with the random effect η_j :

$$P_j = TVP \exp(\eta_j)$$

where TVP is the typical value of the pharmacokinetic parameter P (e.g. CL) in the population, P_j is the individual value for P in the j th individual and η_j is a random variable with a mean of zero and variance ω_p^2 . This model assumes a log normal distribution for the P_j values. Estimates of intersubject variability in P are presented as the square root of ω_p^2 , which is an approximation of the coefficient of variation of P for a log-normally distributed quantity. Random residual variability was modelled according to a combined exponential and additive error model:

$$C_{ij} = C_{ij}^* \exp(\epsilon_{ij,1}) + \epsilon_{ij,4} - \text{for } 5'\text{-DFUR}$$

$$C_{ij} = C_{ij}^* \exp(\epsilon_{ij,2}) + \epsilon_{ij,5} - \text{for } 5\text{-FU}$$

$$C_{ij} = C_{ij}^* \exp(\epsilon_{ij,3}) + \epsilon_{ij,6} - \text{for } \text{FBAL}$$

Figure 1 Basic structural pharmacokinetic model for 5'-DFUR, 5-FU and FBAL [17].

where C_{ij} is the i th plasma concentration measured in the j th individual for the respective metabolite, C^*_{ij} is the respective model-predicted concentration, and the $\epsilon_{ij,k}$ ($k = 1, \dots, 6$) are normally distributed error terms with means of zero and variances σ_k^2 . In the present analysis, the variances for the $\epsilon_{ij,k}$ ($k = 4, 5, 6$) were again fixed to the squared value of the limit of quantification (LOQ) for the corresponding metabolite.

Interoccasion variability (IOV) was evaluated as another, third level of random effects and modelled as

$$P_{jk} = \text{TVP} \exp(\eta_j + \kappa_{jk})$$

where P_{jk} is the individual value for the pharmacokinetic parameter in the j th individual at occasion k , η_j is a random variable with a mean of zero and variance ω_p^2 and κ_{jk} is a random variable with mean of zero and variance π_p^2 at occasion k and zero otherwise.

Data analysis was performed using nonlinear mixed-effect modelling within the program NONMEM (Version 5, level 1). Double precision and first-order estimation were used [21]. All other calculations (e.g. diagnostic graphics, validation) were obtained using SAS (Version 6.12; SAS Institute Inc., Cary, NC, USA) and S-PLUS 2000 software.

In the present investigation, the dose of capecitabine administered on the first day of cycles 2 and 4 was used to estimate PK model parameters for each individual. Individual systemic exposure (i.e. AUC, $C_{\max}$) to 5'-DFUR, 5-FU, and FBAL was then calculated for an intended single capecitabine dose (i.e. 1250 mg m⁻² body surface area; BSA) as previously described [17]. These AUC and $C_{\max}$ values served as actual observations in the concentration-effects analyses described below.

The calculations for exposure rely upon assumptions of linear and time-invariant pharmacokinetics. Preliminary structural PK model building using 5'-DFUR, 5-FU and FBAL as dependent variables utilized phase I data, where a wide range of doses was applied (100–3400 mg) [13]. This analysis revealed nonlinear PK features with dose in the metabolism of 5-FU. In the present analysis capecitabine doses covered a much narrower range (90% of patients received doses between 1500 and 2650 mg). It was therefore assumed that the pharmacokinetic nonlinearity identified in the phase I work could be neglected.

As the population PK model revealed cycle-to-cycle variability on absorption parameters KA and TLAG (see

Table 4), different estimates were calculated for these parameters in any given patient on different occasions. To reduce complexity and to maintain time-invariant kinetics, the mean of individual $C_{\max}$ values was used in the calculations described in the statistical analysis presented here.

Concentration-effect relationships

Independent and dependent variables Concentration-effect analyses were conducted using $C_{\max}$ and AUC of 5'-DFUR, 5-FU and FBAL as six independent variables and selected safety and efficacy outcomes as dependent variables. The dependent safety variables were defined as binary (yes/no) variables and were identified in the analyses as:

- S1 Grade 3–4 adverse events
- S2 Grade 3–4 diarrhoea
- S3 Grade 3 hand-foot syndrome (HFS)
- S4 Grade 3–4 hyperbilirubinaemia (absolute value).

For grade 3–4 adverse events, only treatment-related adverse events were taken into consideration. For diarrhoea and HFS basically all adverse events were assessed as related to treatment and for bilirubin no relationship was assessed by the investigator. Therefore, all events were used for the analysis. Grading of adverse events utilized National Cancer Institute of Canada Clinical Trials Group expanded common toxicity criteria.

The dependent efficacy variables were defined and identified in the analyses as:

- E1 Time to disease progression
- E2 Tumour response/nonresponse
- E3 Duration of survival.

Efficacy variable E2 was defined as a binary variable for response (yes) and nonresponse (no). Time to disease progression and duration of survival were continuous variables measuring the time to the respective event. Deaths were considered as disease progression and were included as such in the analyses. 'Response' was defined as complete or partial tumour response, whereas a 'non-response' was stable or progressive disease. Missing values in response assessment were treated as 'nonresponse'.

Statistical analysis

Univariate logistic regression analysis was performed for all safety variables (S1–S4) and efficacy variable E2. The dependent variable was occurrence (yes/no) of the respective event over the entire study period and the independent variable was systemic exposure 'EXPO' (i.e. AUC or $C_{\max}$) to 5'-DFUR, 5-FU and FBAL. The model is:

$$p = \exp(\alpha + \beta \log[\text{EXPO}]) / (1 + \exp\{\alpha + \beta \log[\text{EXPO}]\})$$

where p is the predicted probability for the occurrence of an event given the independent variable $\log[\text{EXPO}]$, and α and β are the parameters of the model to be estimated from binary data. This analysis was performed under the provision that there were a sufficient number of events for a meaningful analysis.

The Cox proportional hazard model was used in the analysis of time-to-event data (i.e. time to disease progression and duration of survival). The underlying model for the hazard rate was

$$\lambda(t) = \lambda_0(t) \exp(-\beta^T Z)$$

with β being the vector of coefficients of the exposure measures Z , and $\lambda_0(t)$ being the baseline hazard. The Wald test was performed to test any impact of exposure measures. The hypotheses were of the form

$$H_0: \beta_i = 0 \text{ vs } H_1: \beta_i \neq 0$$

with β_i indicating a systemic exposure measure.

As the first step in the analyses, univariate Cox regression was performed with each dependent variable on each of the six independent variables (AUC and $C_{\max}$ of 5'-DFUR, 5-FU, and FBAL). In a second step, multivariate Cox regression was performed where each independent variable which was significant at $P \leq 0.05$ was combined with those covariates which were found to have a significant impact on the particular efficacy parameter in previous clinical subgroup analyses.

Both logistic regression and Cox regression analyses were performed using SAS software.

Results

Population pharmacokinetic model

The statistical comparison of the index and validation data sets showed no significant differences between the data sets for any of the covariates considered in the population pharmacokinetic model. The population PK model for the index data set was successfully validated based on actual drug metabolite concentrations (Table 2), as there was no bias in predictions for 5'-DFUR, 5-FU and FBAL concentrations. Regarding validation based on PK parameters (Table 3), performance between the naive predictor and the population model predictor looked similar, if all data were considered. However, at extreme values of PK parameters (i.e. in the lower and upper deciles of the data), model predictions were better than naive predictions in five out of six cases.

Table 4 shows the results (parameter estimates and corresponding standard errors) of the final population PK model for the complete (index and validation) data set. A rather small value for lag-time (TLAG) combined with large interindividual and interoccasion variability was

Table 2 Summary statistics of (weighted) prediction errors for main capecitabine metabolites.

Metabolite	n	Mean	SD	Min.	Max.	P*
5'-DFUR	781	0.039	1.70	-1.65	40.4	0.521
5-FU	780	0.110	1.78	-2.20	42.9	0.085
FBAL	770	0.004	1.19	-11.23	13.33	0.918

*P is the probability of a greater absolute value of the Student's t -value under the hypothesis that the mean is zero.

Table 3 Predictive performance of the final population PK model for the index data set and for the naive predictor.

Predictor	Bias (mde)		Precision (mae)		Patients*	
	Absolute	%	Absolute	%	Improved	Worsened
5-FU clearance (l h ⁻¹)						
All validation data patients (n = 160)						
Model	-56	-5	185	14	75	85
Naive	-41	-3	157	13		
5-FU CL < 937 (n = 16)						
Model	454	37	454	36	8	8
Naive	478	38	478	38		
5-FU CL > 1509 (n = 16)						
Model	-348	-28	348	28	11	5
Naive	-351	-28	351	28		
CLcr < 50 ml min ⁻¹ (n = 16)						
Model	-51	-5	159	13	5	11
Naive	30	2.4	122	10		
FBAL Clearance (l h ⁻¹)						
All validation set patients (n = 160)						
Model	-0.9	-3.2	4.1	14.8	61	99
Naive	0.2	0.7	2.6	9.2		
FBAL CL < 21.3 (n = 16)						
Model	0.9	4.5	1.5	7.6	15	1
Naive	8.8	31.3	8.8	31.3		
FBAL CL > 35.0 (n = 16)						
Model	-8.7	-30.1	10.5	35.1	10	6
Naive	-9.0	-31.8	9.0	31.8		
CLcr < 50 ml min ⁻¹ (n = 16)						
Model	-6.1	-32.9	6.1	32.9	8	8
Naive	5.2	18.4	5.2	18.4		
FBAL volume (l)						
All validation set patients (n = 160)						
Model	5.3	7.2	8.6	11.6	75	85
Naive	-1.5	-2.2	7.3	10.6		
FBAL volume < 52.8 (n = 16)						
Model	14.6	24.5	14.6	24.5	12	4
Naive	21.0	30.3	21.0	30.3		
FBAL volume > 82.6 (n = 16)						
Model	-8.5	-9.9	13.2	13.6	14	2
Naive	-17.8	-25.7	17.8	25.7		
CLcr < 50 ml min ⁻¹ (n = 16)						
Model	3.1	5.1	5.0	9.2	12	4
Naive	11.3	16.3	13.1	19.0		

*Number of patients with improved (worsened) prediction by the population model compared with the naive predictor. '>' and '<' refer to the lowest and highest deciles.

Table 4 Model parameters of final population PK model for complete data set.

Parameter (unit)	TV	SE (TV)	RE	SE (RE)	Remark
ISV (%CV)					
KA (l h ⁻¹)	1.09	0.166	70	0.166	
TLAG (h)	5.52E-4	2.02E-4	49 498	182 000	
V1 (l)	90.6	14.1	30		ISV fixed
CL1 (l h ⁻¹)	75.8	1.8	24	0.00952	
V2 (l)	17.8		—		TV fixed, no ISV
CL2 (l h ⁻¹)	1190	39.3	33	0.0337	
V3 (l)	73.6	2.35	26	0.0213	
CL3 (l h ⁻¹)	27.5	0.864	32	0.0276	
IOV (%CV)					
IOV KA			70	0.0788	
IOV TLAG			52 915	193 000	
BSAV3: Effect of BSA on V3	0.812	0.189			$V3_{BSA} = V3 (BSA/1.8)^{BSAV3}$
CLRC3: Effect of CLCR on CL3	0.615	0.0769			$CL3_{CLCR} = CL3 (CLCR/80)^{CLRC3}$
CLRV3: Effect of CLCR on V3	0.394	0.109			$V3_{CLCR} = V3 (CLCR/80)^{CLRV3}$
ALPCL2: Effect of ALP on CL2	-0.169	0.0586			$CL2_{ALP} = CL2 (ALP/140)^{ALPCL2}$
RV (%CV)					
Res. Error 5'-DFUR			39†	0.0362	Correlation of ϵ 's for 5'-DFUR and 5-FU: 0.77 (SE: 0.0435)
Res. Error 5-FU			58†	0.0885	
Res. Error FBAL*	$\theta = 0.767$	0.121	34†		

TV, Typical value; SE, standard error of estimate; RE, random effect; ISV, intersubject variability; IOV, interoccasion variability; RV, residual variability; %CV, coefficient of variation (%); KA, first order absorption rate constant; TLAG, lag time; V1, V2, V3, apparent volume of distribution of 5'-DFUR, 5-FU and FBAL, respectively; CL1, CL2, CL3, apparent oral clearance of 5'-DFUR, 5-FU and FBAL, respectively. *The variance of the residual error for FBAL, σ_3^2 , was estimated relative to the variance of the residual error for 5'-DFUR, σ_1^2 , using the equation $\sigma_3^2 = (\theta \times \sigma_1)^2$, where θ is a fixed effects parameter to be estimated. †Values refer to extensive sampling, and values for sparse sampling were estimated to be 30% higher.

Table 5 Statistically significant relationships between covariates and pharmacokinetic parameters in the final model.

Statistically significant effect	Change in independent variable	Effect on the PK parameter	Effect on systemic exposure
ALP on CL of 5-FU	ALP $\times 2$	CL of 5-FU 11% lower	AUC 12% higher
	ALP $\times 5$	CL of 5-FU 24% lower	AUC 31% higher
CLCR on CL of FBAL	CLCR $\times 0.5$	CL of FBAL 35% lower	AUC 53% higher
CLCR on V of FBAL	CLCR $\times 0.5$	V of FBAL 24% lower	C_{max} 41% higher
BSA on V of FBAL	BSA $\times 1.3$	V of FBAL 24% higher	C_{max} 19% lower

estimated. Absorption of capecitabine is highly variable, with some patients presenting lag-times of greater than 4 h, and others having negligible lag-times. Based on decreases in the NONMEM objective function, both TLAG and IOV on TLAG were included in the model. Due to the small population estimate for TLAG, respective random effects had to be rather large to cope with the known variability of the capecitabine absorption process. Overall, model predictions adequately described the data and the diagnostic plots did not indicate any troublesome patterns. The diagnostic plots for individual predicted *vs* observed concentrations are provided in Figure 2.

The main population PK model results are summarized in Table 5. The 'change in independent variable' is

presented as a multiplicative change in the value of the independent variable (covariate) in a direction that would be expected to influence the dependent variable (PK parameter). For example, 'ALP $\cdot 2$ ' denotes a doubling (100% increase) in baseline alkaline phosphatase concentration. Likewise, 'CLCR $\cdot 0.5$ ' denotes a 50% reduction in baseline creatinine clearance. As an example, if patient A has baseline ALP concentration that is twice that of patient B, then the CL of 5-FU is expected to be 11% lower and AUC of 5-FU 12% higher in patient A than in patient B, assuming that all the other characteristics of these two patients are the same.

The effect of creatinine clearance on systemic exposure to FBAL is substantial (50% reduction in CLCR is associated with 35% reduction in CL and 53% increase in

Figure 2 Final population PK model: Diagnostic plots for individual predicted *vs* observed concentrations ($\mu\text{g ml}^{-1}$) (—, lowess scatter plot smooth; ---, line of identity).

AUC of FBAL). This finding was expected, as FBAL is eliminated extensively by renal excretion.

The AUC and C_{max} of 5'-DFUR, 5-FU and FBAL were estimated for each individual from the final population PK model of the complete data set. Respective values are shown in Table 6. Intersubject variability was less for 5'-DFUR and FBAL compared with 5-FU and less for AUC compared with C_{max} . For both 5-FU and FBAL, there was a high correlation between C_{max} and AUC ($r = 0.85$, $P = 0.0001$ for 5-FU, and $r = 0.75$, $P = 0.0001$ for FBAL), but not for any other pair of systemic exposure variables. These PK model-based estimates were then used to define the relationships between concentration and effect.

Relationships between systemic exposure and safety outcomes

The AUC and C_{max} of 5'-DFUR, 5-FU and FBAL broadly overlapped between patients with and without a 'yes' outcome in any of the safety variables (S1–S4). This broad overlap in systemic exposure translated into a small number of statistically significant relationships between systemic exposure and safety outcomes (Table 7). Of the 24 relationships investigated (six measures of exposure and four safety parameters), 21 were not statistically significant. There were three statistically significant relationships, namely a positive association between AUC of FBAL and grade 3–4 diarrhoea ($P = 0.035$), a positive association between AUC of 5-FU and grade 3–4 hyperbilirubinaemia ($P = 0.025$), and a negative association between C_{max} of FBAL and grade 3–4 hyperbilirubinaemia ($P = 0.014$) (Figure 3).

Relationships between systemic exposure and efficacy outcomes

As with the safety analyses, there was a broad overlap in systemic exposure to capecitabine metabolites regardless of efficacy outcomes, which again translated into a small number of statistically significant relationships. Results of the univariate analysis of systemic exposure and investigator-assessed time to disease progression are given in Table 8. Only the AUC of 5-FU was statistically significant in the univariate analysis (hazard ratio (HR) = 1.626, $P = 0.0056$), and this factor remained significant in the multivariate model which included baseline values for age, the predominant site of disease, i.e. liver, number of metastatic sites (one *vs* more), and Karnofsky performance status (70% *vs* other). According to the estimate of the hazard ratio in Table 8, higher exposure (AUC) to 5-FU in plasma leads to higher risk of disease progression. Results of the univariate analysis for duration of survival are given in Table 9. C_{max} of 5'-DFUR was the only significant systemic exposure variable with a positive

Table 6 Descriptive statistics of predicted systemic exposure to 5'-DFUR, 5-FU and FBAL in 481 colorectal cancer patients treated with a single capecitabine dose of 1250 mg m⁻².

Metabolite	Exposure measure (unit)	Mean	%CV	Max.	Q3	Median	Q1	Min.
5'-DFUR	AUC (ug.ml h ⁻¹)	20.48	18	35.04	22.48	20.13	17.89	7.92
	C _{max} (ug ml ⁻¹)	9.18	29	24.71	10.45	8.89	7.58	2.44
5-FU	AUC (ug.ml h ⁻¹)	0.751	41	5.070	0.810	0.707	0.602	0.377
	C _{max} (ug ml ⁻¹)	0.359	80	3.724	0.378	0.299	0.236	0.097
FBAL	AUC (ug.ml h ⁻¹)	25.65	24	57.85	28.56	24.69	21.65	10.79
	C _{max} (ug ml ⁻¹)	4.85	25	11.43	5.46	4.69	4.08	1.83

Table 7 *P*-values (−2 log likelihood statistic) from logistic regression analysis for systemic exposure and safety outcomes (*n* = 481).

Exposure measure	All adverse events (no. of events: 184)	Diarrhoea (no. of events: 59)	Hand-foot syndrome (no. of events: 87)	Hyperbilirubinaemia (no. of events: 115)
5'-DFUR AUC	0.121	0.401	0.065	0.662
5'-DFUR C _{max}	0.199	0.776	0.069	0.127
5-FU AUC	0.503	0.737	0.090	0.025
5-FU C _{max}	0.864	0.957	0.599	0.832
FBAL AUC	0.144	0.035	0.384	0.468
FBAL C _{max}	0.543	0.365	0.355	0.014

Table 8 Cox regression analysis for systemic exposure and time to disease progression.

Exposure measure	Hazard ratio	95% CI (Wald)	<i>P</i> -value (Wald)
5'-DFUR AUC	0.996	0.97–1.02	0.75
5'-DFUR C _{max}	0.965	0.93–1.00	0.068
5-FU AUC	1.626	1.15–2.29	0.0056
5-FU C _{max}	1.097	0.72–1.67	0.66
FBAL AUC	0.989	0.97–1.01	0.16
FBAL C _{max}	0.918	0.84–1.01	0.063

n = 481, 426 events.

association with survival (HR = 0.938, *P* = 0.0048). C_{max} of 5'-DFUR remained significant in the multivariate analysis which included baseline values for the presence of multiple liver metastasis, number of metastatic sites (one *vs* more), Karnofsky performance status, and centre as covariates.

For further investigation of the clinical relevance of these findings, values for AUC of 5-FU and C_{max} of 5'-DFUR were divided into quartiles. Respective Kaplan–Meier plots for the first, second and third (combined), and fourth quartiles are presented in Figure 4. For the AUC of 5-FU, there was a difference of 19 days in median time to disease progression between the first and fourth quartiles, suggesting that systemic exposure to 5-FU has no effect on time to disease progression. For the C_{max} of 5'-DFUR, patients in the first quartile had a

Table 9 Cox regression analysis for systemic exposure and duration of survival.

Exposure measure	Hazard ratio	95% CI (Wald)	<i>P</i> -value (Wald)
5'-DFUR AUC	0.993	0.96–1.03	0.65
5'-DFUR C _{max}	0.938	0.90–0.98	0.0048
5-FU AUC	1.248	0.98–1.59	0.073
5-FU C _{max}	1.009	0.70–1.46	0.96
FBAL AUC	1.001	0.98–1.02	0.91
FBAL C _{max}	0.922	0.83–1.02	0.11

n = 481, 296 events.

median survival of 355 days, whereas the patients in the second and third, and fourth quartiles showed median survival of 423 and 458 days, respectively.

Discussion

There was a broad overlap in systemic exposure to capecitabine metabolites in patients regardless of the safety and efficacy outcomes. In view of the large number of patients (*n* = 481) and events in these analyses, it is doubtful that a lack of statistical power is an explanation for the small number of statistically significant relationships. *P*-values inferring statistically significant effects for safety outcomes were quite large (*P* > 0.01). Significant *P*-values (*P* < 0.05) may have also been the result of multiple hypothesis testing. Owing to the exploratory

Figure 3 Statistically significant logistic regression relationships between systemic exposure and safety measures (—, model prediction; ---, upper and lower 95% confidence intervals; x, observations (0, no event, 1, event)). (a) AUC of FBAL *vs.* diarrhoea; (b) AUC of FBAL *vs.* hyperbilirubinaemia; (c) $C_{\max}$ of FBAL *vs.* hyperbilirubinaemia.

nature of the analysis, no adjustment for multiplicity was done. The weak relationships between systemic exposure and safety and efficacy outcomes may be due to the fact that concentrations of the different metabolites in plasma do not necessarily reflect these in the organs and tissues where adverse events and tumour response occur.

The poor relationship between systemic exposure and safety distinguishes oral capecitabine from intravenous 5-FU and other chemotherapeutic agents. After administration of 5-FU, 5-FU concentrations in plasma are a reasonable marker of the exposure of tissues to the drug. Thus, with intravenous 5-FU administration, the relationship between systemic exposure and safety outcome are well established and are reported consistently [2–6].

Figure 4 (a) Estimated probability for time to disease progression classified by the AUC of 5-FU (----, 1st; - - - -, 2nd and 3rd; —, 4th quartile). (b) Estimated probability for duration of survival classified by the $C_{\max}$ of 5'-DFUR (----, 1st; - - - -, 2nd and 3rd; —, 4th quartile).

After administration of capecitabine, 5-FU concentrations in plasma do not reflect the exposure of tissues to 5-FU because of the presence of the activation enzymes in tissues. Capecitabine was designed to limit plasma concentrations of 5-FU by allowing 5-FU to be selectively synthesized in the tumour. Numerous studies investigating the relationship between pharmacokinetics (e.g. plasma concentration, AUC, $C_{\max}$, steady-state concentrations) and toxicity for many other cancer chemotherapeutic agents have been reported [7–10]. One recent report used an approach similar to the one described herein, namely a first estimate of the pharmacokinetic parameters for each patient based on the population model and Bayesian estimation techniques, and then the determination of whether a pharmacokinetic–pharmacodynamic relationship exists using logistic and

Cox regression [9]. This report showed that some pharmacokinetic parameters (e.g. clearance) for docetaxel were a predictor of haematological toxicity.

One of the few statistically significant relationships between systemic exposure to capecitabine and safety outcomes was a positive relationship between AUC of FBAL and occurrence of grade 3–4 diarrhoea. In a logistic regression analysis, AUC of FBAL was a significant predictor of grade 3–4 diarrhoea. However, this result was not consistent when analysing the two phase III studies separately. The lack of statistical significance in the individual studies and the broad overlap in systemic exposure to FBAL between patients with and without grade 3–4 diarrhoea raise doubts about the clinical significance of these findings. A statistically significant association between exposure to FBAL and diarrhoea does not necessarily indicate that FBAL causes diarrhoea. It is more likely that 5-FU, rather than FBAL, causes diarrhoea because it is cytotoxic whereas FBAL is not. Since FBAL is the main and final catabolite of 5-FU, it is possible that systemic exposure to FBAL, as measured by plasma concentrations, may be a marker for the amount of 5-FU that was formed in tissues.

Two divergent relationships were found between systemic exposure to capecitabine metabolites and occurrence of grade 3–4 hyperbilirubinaemia. There was a positive association with AUC of 5-FU and a negative association with $C_{\max}$ of FBAL. Since 5-FU is the active metabolite and hyperbilirubinaemia has been reported after administration of other fluoropyrimidines [22], such a relationship with AUC of 5-FU may be expected. The relationship for the $C_{\max}$ of FBAL is more difficult to interpret because of its negativity. One possible explanation could be that a higher $C_{\max}$ for FBAL represents a faster rate constant for the formation of FBAL, because of a more rapid catabolism of 5-FU. With less 5-FU there is less chance of toxicity, which would result in a lower probability of hyperbilirubinaemia. The findings from the combined data were consistent with the results seen for each study independently.

Although hand–foot syndrome is the most frequently encountered specific adverse event following capecitabine treatment (data on file), there were no statistically significant relationships between systemic exposure to capecitabine metabolites 5'-DFUR, 5-FU and FBAL and grade 3 hand–foot syndrome. This finding is consistent with the results for all grade 3–4 adverse events.

The weak relationships seen between systemic exposure to capecitabine metabolites and efficacy outcomes are not inconsistent with those seen with intravenous 5-FU. Whether administered by this route or via a pro-drug, the concentration of 5-FU in the tumour depends on its thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase (DPD) activity. Thymidine phos-

phorylase is responsible for the metabolism of 5'-DFUR to the active moiety 5-FU, and DPD is responsible for catabolizing 5-FU. This has been demonstrated in animals by strong relationships between efficacy and the TP : DPD ratio [23]. Following administration of intravenous 5-FU, a positive relationship between systemic exposure and efficacy was found in only two [24, 25] out of five studies [24–28]. Only one report explored the relationship between systemic exposure and survival and the positive correlation was not statistically significant [25].

The main finding for systemic exposure to capecitabine metabolites and efficacy outcomes was a statistically significant positive relationship between the $C_{\max}$ of 5'-DFUR and survival. This finding was consistent in the analysis of the combined data and in each phase III study, which suggests that it is a stable and reliable result. The relationship may be explained by 5'-DFUR in plasma, as the immediate precursor of the active agent 5-FU, best reflecting the extent of exposure of the tumour to 5-FU. However, no relationships were found between $C_{\max}$ of 5'-DFUR and other efficacy parameters (time to disease progression, tumour response), which weakens this hypothesis.

The existence of such a relationship for the $C_{\max}$ of 5'-DFUR but not for the AUC of 5'-DFUR is unexpected. In the area of oncology, efficacy generally correlates better with AUC rather than $C_{\max}$ [29]. *In vitro* studies investigating the cell-killing effect of 5-FU have shown that 5-FU is an 'AUC-dependent drug'. Short exposure requires high concentrations to obtain the cell-killing effect, whereas the same effect can be obtained with longer exposure to lower concentrations [30]. Furthermore, experiments in mice showed similar antitumour activity when the same daily dose of capecitabine was administered once or twice daily [23]. Assuming dose-proportional pharmacokinetics in mice, once-daily dosing should have produced a $C_{\max}$ twice as high as the twice-daily dosing regimen with the same AUC. Therefore, this suggests that AUC, and not $C_{\max}$, correlates with antitumour activity in mice. The significant concentration–effect relationship with the $C_{\max}$ of 5'-DFUR but not the AUC may be the consequence of the respective ranges for both measures of systemic exposure. The range was greater for $C_{\max}$ (max/min = 10.1) than for AUC (max/min = 4.4). From a statistical viewpoint, a significant relationship will be more readily detected with a broader range of the independent variable, and this may explain our finding. Thus, our results do not imply that capecitabine should be dosed once daily to increase $C_{\max}$ and therefore survival.

The relationship between time to disease progression and the AUC of 5-FU was found to be significant with a negative association. There are three possible explana-

tions for this finding. First, it is possible that patients for whom capecitabine is most effective are patients with marked tumour selectivity and increased trapping of 5-FU. This would lead to lower concentrations of 5-FU in plasma and therefore would explain our negative relationship between systemic exposure and efficacy. Results obtained after administration of intravenous 5-FU support this hypothesis. Presant *et al.* [31] showed that trapping of 5-FU in the tumour is highly predictive ($P = 0.000\ 021$) of drug response. Trapping was defined as intratumoral half-life of 20 min or more. Because plasma concentrations of 5-FU were not obtained in that study, our explanation of a simultaneous increase of 5-FU in the tumour and a decrease of 5-FU in plasma cannot be fully supported.

A second possible explanation may lie in a confounding effect of serum alkaline phosphatase (ALP) concentrations at baseline. This was a strong predictor of poor survival after treatment with capecitabine (univariate Cox regression, $P = 0.0001$) and is thus highly correlated with a dependent efficacy variable in the present analysis. Baseline serum ALP is also highly correlated with the AUC of 5-FU (Table 5), which is an independent variable. Therefore serum ALP is highly correlated with both an independent and dependent variable in the efficacy analyses. Patients with high ALP at baseline have a shorter time to disease progression and also a higher AUC of 5-FU. To explore the influence of both ALP and the AUC of 5-FU on time to disease progression, a bivariate Cox regression was performed with the factors AUC of 5-FU and ALP at baseline. Using this model, the relationship with ALP remained significant ($P = 0.0007$) and that with the AUC of 5-FU became statistically nonsignificant ($P = 0.056$). An identical bivariate analysis was performed for tumour response and survival giving similar results. These findings support the second explanation that serum ALP at baseline confounds the relationships between AUC of 5-FU and the efficacy parameters. This illustrates the importance of including patient characteristics that are associated with PK parameters when modelling pharmacodynamic relationships.

A third possible explanation would be a combination of the first two, since they are not mutually exclusive. Because these negative relationships appear to be driven by extraneous and potentially confounding factors, the results should not be interpreted as suggesting that the dose of capecitabine should be lowered to increase efficacy.

In the pharmacokinetic model, $C_{\max}$ and AUC were calculated for an intended single dose of capecitabine on the basis of individual PK parameters. Despite similar $C_{\max}$ and AUC values after a single dose, it is possible that the cumulative AUC may differ considerably between patients, depending on the number of courses

of treatment or the doses themselves. However, previous analyses of data from phase I studies showed that AUC and $C_{\max}$ after a single dose correlate better with safety than cumulative AUC [13]. In the present study, adverse events and response to treatment mainly occurred with the first two cycles (6 weeks) when one-third of patients stopped treatment. Therefore, a consideration of cumulative AUC was not informative. An alternative approach would be to consider individual dosing regimens. However, this would require a considerably more elaborate exposure–response model including time as factor.

A related potential limitation is that AUC and $C_{\max}$ reflect exposure prior to a potential dose reduction for toxicity. A PK/PD model that could also describe the time course of the PD variable (e.g. intensity of side-effect *vs* time, or size of the tumour *vs* time) would be better, but this approach was not feasible due to the complexity of modelling the PK of three metabolites and the PD outcomes (tumour response or side-effects) over time. To our knowledge, there are no reports describing such a PK/PD model in the field of oncology with phase 3 data. An attempt to model both PK and safety aspects has been made with paclitaxel myelosuppression [10], and to describe the PK of capecitabine using a physiological approach [32]. The complexity of the model and the number of parameters limit the utility of this work in the context of sparse data obtained in patients.

In conclusion, the results show that there are broad overlaps in systemic exposure to capecitabine metabolites for all patients regardless of safety and efficacy outcomes. These overlaps translate into absent or weak relationships between systemic exposure and safety/efficacy parameters. The basis of these findings may be that concentrations of 5-FU, 5-DFUR and FBAL in plasma do not necessarily reflect concentrations in healthy tissues and tumours after capecitabine therapy. Our data suggest that plasma concentrations of immediate precursors or catabolites of the active moiety may reflect its tissue concentrations, but this requires further investigation.

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