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Pharmacokinetic profile of cetuximab (Erbitux™) alone and in combination with irinotecan in patients with advanced EGFR-positive adenocarcinoma

Catherine Delbaldo [*,a], Jean-Yves Pierga [b], Véronique Dieras [b], Sandrine Faivre [a], Valérie Laurence [b], Jean-Claude Vedovato [c], Marc Bonnay [a], Matthias Mueser [c], Arno Nolting [c], Andreas Kovar [c], Eric Raymond [a,*]

[a] *Department of Medicine and Department of Clinical Biochemistry, Institut Gustave-Roussy, Villejuif, France*
[b] *Institut Curie, Paris, France*
[c] *Merck KGaA, Darmstadt, Germany*

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Abstract

This trial assessed pharmacokinetic interactions between cetuximab and irinotecan.

Patients were placed in either in group A (irinotecan 350 mg/m^2/3 weeks and 400 mg/m^2 cetuximab at week 2 then 250 mg/m^2/week) or group B (cetuximab weekly starting week 1 then irinotecan starting week 4). Patient plasma or serum samples from each treatment arm were analysed using HPLC and ELISA. Among 14 patients, compartmental model showed no significant differences in mean plasma AUC at week 1 *versus* week 4 for irinotecan (44,388 *versus* 39,800 μg/ml/h) and cetuximab (20,441 *versus* 23,363 μg/ml/h), respectively. Half-lifes (standard deviations) for irinotecan were 16.02 (±8.41) h at week 1 and 13.99 (±2.14) h at week 4, and for cetuximab 106 (±32) at week 3 and 111 (±30) h at week 4. Mean concentration-*versus*-time profiles either alone or in combination were superimposable for cetuximab and irinotecan. From this study, we conclude that there is no evidence of pharmacokinetic interaction between irinotecan and cetuximab.

Keywords: EGFR inhibitor; C225; Colon cancer; Phase I

1. Introduction

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein [1–4] that is frequently expressed or mutated in a broad range of malignancies and has been proposed as a therapeutic target for cancer treatment [5–8]. Cetuximab (Erbitux™) is a novel IgG1 monoclonal antibody that binds EGFR with high specificity and affinity [9]. Binding of cetuximab to EGFR prevents ligand-induced EGFR phosphorylation and activation of the kinase domain and inhibits the growth of EGFR-driven human cancer cells [10,11]. In preclinical studies, concentrations of $\geqslant$2 nM cetuximab induced a significant reduction of tumoural volume in a variety of human cancer models [12,13]. Phase I studies with cetuximab alone showed moderate skin toxicity and hypersensitivity reactions [14,15]. At doses ranging 200–400 mg/m^2, cetuximab displayed linear predictable pharmacokinetics and a clearance value of approximately 0.02 L/h/m^2. Phase III study confirmed

* Corresponding author. Present address: Service Inter-Hospitalier de Cancérologie Bichat-Beaujon, Hôpital BEAUJON, 100 boulevard du Général Leclerc, 92110 Clichy Cedex, France. Tel.: +33 1 4087 5617; fax: +33 1 4087 5487.
E-mail address: eric.raymond@bjn.ap-hop-paris.fr (E. Raymond).

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acceptable tolerance and showed a high level of disease control [16].

Irinotecan demonstrated significant survival benefits in patients with colorectal cancer [17,18]. Irinotecan has a complex metabolism requiring activation into SN-38 by carboxylesterase [19] and glucuroconjugation for catabolism [20]. Preclinical studies demonstrated additive and synergistic activity between topisomerase I inhibitors and cetuximab [21,22]. Clinical trials combining irinotecan with cetuximab were conduced in patients with colorectal cancer who failed a first line treatment with single agent irinotecan, yielding 20–25% objective responses and strongly suggested that the addition of cetuximab might overcome the resistance to irinotecan [23,16]. Based on these results, the combination of cetuximab with irinotecan was approved for the treatment of patients with advanced EGFR-positive colon carcinoma and has prompted us to investigate the potential pharmacokinetic interactions between the two drugs.

2. Patients and methods

2.1. Inclusion criteria

Patients with EGFR expression in tumours (DAKO Cytomation, Glostrup, Denmark) meeting the following inclusion criteria were eligible: adenocarcinoma; age $\geqslant$18 years; Karnofsky performance status $\geqslant$60%; body mass index (BMI) between 18 and 30 kg/m^2; adequate bone marrow function (leukocyte count $\geqslant 3.0 \times 10^9$/L, absolute neutrophil count $\geqslant 1.5 \times 10^9$/L, platelets $\geqslant 100 \times 10^9$/L and hemoglobin $\geqslant$8 g/dL); serum creatinine value $1.5 \times \leqslant$the upper limit of normal (ULN); total bilirubin level $\leqslant 1.5 \times$ ULN that had no increased by >25% over the preceding 4 weeks; ASAT and ALAT $\leqslant 5 \times$ ULN; no chemotherapy, radiotherapy or surgery (excluding biopsy) within 4 weeks before study entry; no intercurrent history of uncontrolled severe diseases; no concomitant experimental new drug; no previous exposure to monoclonal antibody therapy and signed informed consent.

2.2. Study design

The first six patients were assigned to group A until the primary endpoint was reached, and subsequent patients were assigned to group B.

In group A, the schedule was designed to investigate the effects of cetuximab on pharmacokinetic parameters of irinotecan and its metabolites. Patients received irinotecan at week 1 followed by cetuximab at week 2.

In group B the treatment was scheduled to detect the effect of irinotecan on pharmacokinetic parameters of cetuximab. Patients received cetuximab at week 1 followed by irinotecan at week 4. For both groups, the pharmacokinetic interaction was studied during the first 4 weeks and the combination was monitored until disease progression or unacceptable toxicity.

2.3. Pretreatment and follow-up examinations

Complete medical history, physical examination, laboratory tests (complete blood count, creatinine, serum electrolytes, calcium, uric acid, total protein, albumin level, hepatic and coagulation tests, and LDH) and urinalysis were performed at baseline and repeated weekly.

Toxicity was evaluated weekly and graded using the National Cancer Institute's Common Toxicity Criteria (NCI-CTC), version 2.0. Tumours were evaluated and/or measured at baseline and reassessed every two months, using the World Health Organization standard criteria.

2.4. Drug administration

Cetuximab (prepared by Imclone Systems Inc. as 50 ml ready-to-use vials containing 100 mg of cetuximab, 2 mg/ml) was administrated as a 120-min intravenous infusion at an initial dose of 400 mg/m^2 followed by a weekly dose of 250 mg/m^2, given as a 1-h infusion with prophylactic intravenous dexchlorpheniramine maleate.

Irinotecan was given at a dose of 350 mg/m^2 as a 60-min intravenous infusion every 21 days with adequate standard antiemetic regimens. Delayed diarrhea was treated with loperamide and antibiotics when associated with grades 3 and 4 leucopenia and/or fever. When combined, cetuximab was given prior to irinotecan with at least 1 h wash out period.

The schedule of treatment, dose and infusion time was not supposed to be modified during the 4-weeks pharmacokinetic phase. If adjustments were made for safety reasons, the patient was considered not evaluable for pharmacokinetic analysis.

2.5. Pharmacokinetic analysis

2.5.1. Pharmacokinetics of irinotecan (group A)

Plasma samples were collected at weeks 1 and 4; before the start of irinotecan infusion, at 1, 2, 3, 6, 10, 24, and 48 h after. Whole blood (2.5 ml) samples were collected in heparinised tubes and centrifuged within 30 min at 2500*g* for 45 min at 4 °C, 250 µl of plasma was transferred into ice-cold tubes and stored at −80 °C, before analysis.

Quantitative analysis of irinotecan and its metabolites was performed using a reverse-phase high-performance liquid chromatography with fluorescence detection (HPLC) as described elsewhere [24,25]. The lower limit

of quantification (LLQ) was 5 ng/ml for irinotecan and 10 ng/ml for SN-38.

2.5.2. Pharmacokinetics of cetuximab (group B)

Serum samples were collected at week 1: before start of cetuximab infusion and 2 h after; at week 2: before start of infusion and 1 h after; at week 3: before start of infusion and 1, 2, 6, 10, 24, 48, 96 h after; at week 4: before start of infusion and 1, 2, 6, 10, 24, 48, 96 and 168 h after. Whole blood (2.5 ml) samples were collected in vacuum tubes. Serum was prepared and dispatched to the central laboratory for analysis (Institute of Pharmacokinetics and Metabolism, Merck KGaA, Grafing, Germany).

The bioanalytical methodology involved a sandwich enzyme-linked immunosorbent assay (ELISA) that was developed and validated for the determination of cetuximab in human serum samples by the Institute of Drug Metabolism and Pharmakinetics (Merck KGaA, Grafing, Germany). After incubation, plates were washed with buffer and further incubated with rabbit anti-human IgG conjugated with horseradish peroxidase, which bound to cetuximab. Unbound conjugate was removed by the addition of tetramethylbenzidine (TMB), a substrate for horseradish peroxidase, inducing an enzymatic reaction. The intensity of the reaction was measured by a microtiter plate reader at 450 nm and was used to determine the cetuximab serum sample concentration. The LLQ for cetuximab was 100 ng/ml.

Maximum plasma concentration, area under the plasma/serum concentration time curve (AUC) and terminal half-life were determined by non-compartmental and compartmental analysis for irinotecan and cetuximab and non-compartmental analysis for SN-38, using the Kinetica software program version 4.0 (InnaPhase Corporation, Philadelphia, PA, USA).

2.6. Immunology

Patient serum samples were evaluated for the presence of antibodies directed against cetuximab (HACA) before each administration of cetuximab and at the end of the study.

3. Results

3.1. Patient characteristics

A total of 40 patients were screened and 75% were found to be EGFR-positive. Twenty-six patients were not eligible while 14 patients received the study medication (six in group A and eight in group B, Table 1). All patients but one completed the pharmacokinetic program and were assessable for safety and anti-tumour activity (six patients in group A and seven patients in group B). One patient entered in group B had to discontinue treatment after two doses of cetuximab due to early evidence of disease progression. A total of 66 cycles of treatment were administrated (median number of cycles: 3.5, range 1–11).

Table 1
Patient characteristics at baseline

Number of patients	Group A ($n = 6$)	Group B ($n = 8$)	Total ($n = 14$)
Age (years)			
Median	55.5	52	54
Range	24–67	39–62	24–67
Sex			
Male/female	3/3	3/5	6/8
Karnofsky index			
100	2	1	3
90	0	0	0
80	4	7	11
Primary tumour			
Cardia	0	1	1
Caecum	1	0	1
Appendix	0	1	1
Colon	4	3	7
Rectum	0	2	2
Pancreas	1	0	1
Prostate	0	1	1
No. of metastatic sites			
1	2	0	2
2	2	5	7
3	1	3	4
$\geqslant 4$	1	0	1
Site of disease			
Liver	5	7	12
Lung	5	6	11
Peritoneum	0	1	1
Bone	1	2	3
Lymph nodes	1	3	4
Ovary	1	0	1
Median no. of prior chemotherapy [range]	4 [3–5]	3 [2–10]	4 [2–10]
Previous chemotherapy			
With irinotecan	5	6	11
Without irinotecan	1	2	3
Previous surgery	6	7	13
Previous radiotherapy	1	1	2

3.2. Pharmacokinetics

In group A, irinotecan was analysed using a non-compartmental (Table 2) and a bi-compartmental analysis (Table 3) and the best fit was obtained with the bi-compartmental analysis. The pharmacokinetic parameters obtained when irinotecan was given alone or in combination with cetuximab demonstrated no significant difference, indicating that cetuximab had no impact on the pharmacokinetics of irinotecan. Mean AUCs for irinotecan were $42{,}792 \pm 22{,}277$ μg/ml/h at week 1 and $39{,}051 \pm 16{,}852$ μg/ml/h at week 4 in the

Table 2
Main pharmacokinetic parameters of irinotecan, SN-38, and cetuximab: non-compartmental analysis

No. of patients	Group A ($n = 6$)				Group B ($n = 7$)	
	Irinotecan		SN-38		Cetuximab	
	Week 1 (without cetuximab)	Week 4 (with cetuximab)	Week 1 (without cetuximab)	Week 4 (with cetuximab)	Week 3 (without irinotecan)	Week 4 (with irinotecan)
AUCτ (ng/ml/h)						
Mean (SD)	42,792 (22,277)	39,051 (16,852)	931 (948)	609 (531)	13,039 (4783)	14,923 (5029)
[range]	24,651–77,637	20,366–69,746	317–2754	215–1487	6234–19,019	8918–22,386
C max (ng/ml)						
Mean (SD)	8129 (2882)	6783 (1293)	88 (26)	57 (20)	153 (38)	162 (43)
[range]	5150–13,407	5095–8799	55–121	23–83	112–225	115–225
Half-life (h)						
Mean (SD)	10 (3)	10 (2)	23 (35)	15 (23)	119 (42)	117 (32)
[range]	7–13	7–12	2–93	2–61	82–188	85–173
V_{dss} (L)						
Mean (SD)	144 (41)	147 (22)	–	–	3.59 (0.81)	3.29 (0.86)
[range]	108–222	120–172			2.24–4.52	1.88–4.29
Clearance (L/h)						
Mean (SD)	16.6 (6.6)	17 (6.2)	–	–	0.035 (0.009)	0.032 (0.01)
[range]	8.2–22.6	8.4–27.1			0.022–0.044	0.019–0.045

Table 3
Main pharmacokinetic parameters of irinotecan and cetuximab: compartmental analysis

No. of patients	Group A ($n = 6$)		Group B ($n = 7$)	
	Irinotecan		Cetuximab	
	Week 1 (without cetuximab)	Week 4 (with cetuximab)	Week 3 (without irinotecan)	Week 4 (with irinotecan)
AUC (μg/ml/h)				
Mean (SD)	44,388 (24,678)	39,800 (18,856)	20,441 (9688)	23,363 (9395)
[range]	22,929–83,190	18,955–74,177	11,765–37,020	11,240–34,677
C max (μg/ml)				
Mean (SD)	7596 (3269)	6110 (1151)	130 (31)	143 (40)
[range]	5257–14,028	4069–7361	101–180	106–199
Half-life (h)				
Mean (SD)	16.02 (8.41)	13.99 (2.14)	106 (32)	111 (30)
[range]	9.56–31.52	9.88–15.95	79–160	73–158
V_{dss} (L)				
Mean (SD)	175 (61)	167 (36)	3.48 (0.7)	3.2 (0.73)
[range]	104–273	138–230	2.35–4.14	2.13–3.8
Clearance (L/h)				
Mean (SD)	16.9 (7.2)	17.5 (7)	0.025 (0.0)	0.022 (0.009)
[range]	8–24.4	8.4–29.3	0.011–0.03	0.012–0.035

Irinotecan: two-compartmental analysis; cetuximab: one-compartmental analysis.

non-compartmental model (Table 2). In the bi-compartmental model, mean AUCs were 44,388 ± 24,678 μg/ml/h at week 1 and 39,800 ± 18,856 μg/ml/h at week 4 (Table 3). The half-life of irinotecan was 16.02 ± 8.41 h at week 1 and 13.99 ± 2.14 h at week 4 in a compartmental model (Table 3) and 10 ± 3 h at week 1 and 10 ± 2 h at week 4 in a non-compartmental model (Table 2). Mean irinotecan concentration-*versus*-time profiles either alone or in combination with cetuximab were superimposable (Fig. 1(a)).

As previously described, large interpatient variability was observed for SN-38 [27]. Mean SN-38 pharmacokinetic parameters obtained by non-parameter analysis, when irinotecan was given alone or in combination, displayed small differences that were not statistically different (Tables 2 and 3). As shown in Fig. 1(b), mean concentration-*versus*-time profiles of SN-38 between weeks 1 and 4 were nearly superimposable. This indicated that the combination of irinotecan and cetuximab had no effect on extent of conversion of irinotecan into its active metabolite.

In group B, cetuximab was analysed using a non-compartmental and a mono-compartmental approach. The best fit was obtained with a mono-compartmental model. As shown in Tables 2 and 3, the comparison of the mean pharmacokinetic parameters of cetuximab before and

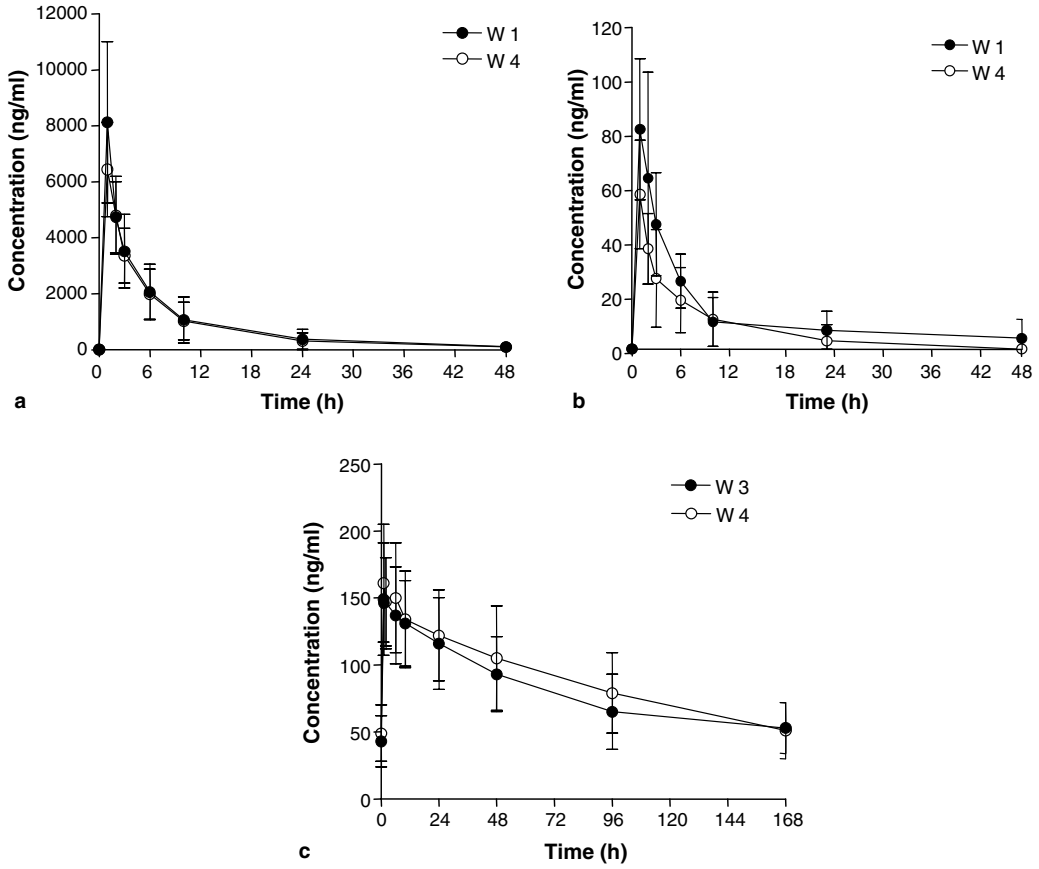

Fig. 1. Mean concentration-*versus*-time curves of (a) irinotecan, (b) SN-38, (c) cetuximab.

after the administration of irinotecan (weeks 3 and 4) displayed no difference, suggesting the absence of pharmacokinetic interaction with irinotecan. Mean AUCs of cetuximab were 13,039 ± 4783 μg/ml/h at week 3 and 14,923 ± 5092 μg/ml/h at week 4, in a non-compartmental model (Table 2) and 20,441 ± 9688 μg/ml/h at week 3 and 23,363 ± 9395 μg/ml/h at week 4, in a compartmental model (Table 3). The half-life of cetuximab was 106 ± 32 at week 3 and 111 ± 30 h at week 4 in a compartmental model (Table 3) and 119 ± 42 at week 3 and 117 ± 32 h at week 4, in a non-compartmental model (Table 2). Administration of irinotecan with cetuximab had no influence on the half-life of cetuximab. A concentration-*versus*-time curve indicates that the presence of irinotecan had no significant impact on the concentration of cetuximab (Fig. 1(c)).

3.3. Immunology

No antibody directed against cetuximab (HACA) was detected in the sera of patients.

3.4. Safety

In group A, grade 3 anemia occurred in one patient requiring blood transfusion. Another patient experienced a grade 3 neutropenia without infection. In group B, one patient experienced two episodes of grade 4 non-febrile neutropenia and a grade 3 anemia. Skin toxicity included acneiform-follicular rashes on the scalp, face and trunk; macropapular eruptions and dry skin. Grades 1 and 2 skin toxicity was reported for 11 patients (five in group A and six in group B) and grades 3 and 4 for one patient in each group. Grades 3 and 4 diarrhea occurred in two patients in group B. Data for each group showed no significant increase in grades 3 and 4 toxicities for the combination as compared to the single agent therapy.

3.5. Response

The median duration of tumour stabilisation was 10 weeks (range 4–33) with prolonged tumour stabilisations reported in one patient (24 weeks) in group A and two patients (32 and 33 weeks) in group B.

4. Discussion

Pre-clinical studies in colon cancer showed that cytotoxicity, apoptotic cell death, and clinical activity induced by a topoisomerase I inhibitors were enhanced by the addition of cetuximab [22,23]. Moreover, the

combination of cetuximab with irinotecan was shown to reverse clinical resistances in patients progressing on single agent irinotecan [16,23]. Drug interactions with irinotecan have been previously described [26] prompting us to identify potential pharmacokinetic interactions between cetuximab and irinotecan in patients with advanced EGFR-expressing adenocarcinoma.

Among the 40 screened patients, 75% had EGFR-expressing adenocarcinoma, which is in good agreement with the results of previous trials [16,23,28]. Early phases II and III clinical trial results that explored cetuximab and irinotecan combination, showed that the main toxicities of irinotecan, in particular delayed diarrhea and neutropenia, were not increased by the addition of cetuximab. In terms of safety, the majority of adverse events observed in our study were either considered to be due to the underlying disease or consistent with the known safety profiles of cetuximab and irinotecan [16,23,28,29]. Although the number of patients entered in this trial remained limited, no change in toxicity rate was detectable in patients treated with the combination compared to the single agent therapy.

Our results demonstrated that the addition of cetuximab to irinotecan had no impact on the drug disposal of irinotecan and its metabolites. We did not show any significant difference in mean AUCs, Cmax and clearance of irinotecan after the addition of cetuximab. As expected and reported previously [27], we observed a larger interpatient variability for SN-38 compared to irinotecan, without significant difference when cetuximab was added to irinotecan. The addition of cetuximab to irinotecan did not seem to change the extent of conversion of irinotecan into SN-38. We have also demonstrated that the addition of irinotecan to cetuximab did not alter the pharmacokinetic profile and drug disposal of cetuximab. The exploration of pharmacokinetic parameters did not provide any explanation for the observed supra-additive pre-clinical activity and the clinical efficacy of the combination of irinotecan and cetuximab.

In summary, from our data no significant pharmacokinetic interaction was observed between irinotecan and cetuximab, suggesting that the clinical anti-tumour activity of irinotecan and cetuximab combination is not likely to be related to pharmacokinetics. The absence of pharmacokinetic interaction (*i.e.*, the non-overlapping toxicity between the two drugs) has been recognised to safely promote further registration phases II–III trials.

Conflict of interest statement

Jean-Claude Vedovato, Matthias Mueser, Arno Nolting, and Andreas Kovar are employed by Merck KGaA. Other authors have declared no conflict of interest.

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