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Pharmacokinetics Evaluation of Nimotuzumab in Combination with Doxorubicin and Cyclophosphamide in Patients with Advanced Breast Cancer

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Abstract: EGFr (Epidermal growth factor receptor) overexpression has been detected in many tumors of epithelial origin, specifically in breast cancer and it is often associated with tumor growth advantages and poor prognosis. The nimotuzumab is a genetically engineered humanized MAb (monoclonal antibody) that recognizes an epitope located in the extracellular domain of human EGFr. The aim of this study was to assess the pharmacokinetics of nimotuzumab in patients with locally advanced breast cancer who are receiving neoadyuvant therapy combined with the AC chemotherapy regimen (i.e., 60 mg/m^2 of Doxorubicin and 600 mg/m^2 of Cyclophosphamide in 4 cycles every 21 days). A single center, non-controlled, open Phase I clinical trial, with histopathological diagnosis of locally advanced stage III breast cancer, was conducted in 12 female patients. Three patients were enrolled at each of the following fixed dose levels: 50, 100, 200 and 400 mg/week. Multiple intermittent short-term intravenous infusions of nimotuzumab were administered weekly, except on weeks 1 and 10, when blood samples were drawn for pharmacokinetic assessments. Nimotuzumab showed dose-dependent kinetics. No anti-idiotypic response against nimotuzumab was detected in blood samples of participants. There was not interaction between the administration of nimotuzumab and chemotherapy at the dose levels studied. The optimal biological doses ranging were estimated to be 200 mg/weekly to 400 mg/weekly.

Key words: Breast cancer, epidermal growth factor receptor, monoclonal antibody, nimotuzumab, pharmacokinetics.

1. Introduction

The HER (human epidermal growth factor receptor) family consists of four tyrosine kinase receptors: HER1/ErbB-1 (epidermal growth factor receptor (EGFr)), HER2/ErbB-2/ Neu, HER3/ErbB-3 and HER4/ErbB-4 [1]. These receptors are highly expressed in many solid tumor types, including breast [2], lung [3], ovarian [4], colorectal [5] and prostate [6]. They also play an important role in the proliferation, differentiation, motility, adhesion, protection from apoptosis and transformation of tumor cells [1, 7, 8].

Several strategies have been developed to disrupt the EGFr-associated signal transduction cascade. The main therapeutic approaches include MAb (monoclonal antibodies) [8, 9] directed against the extracellular binding domain of the receptor and small

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molecule tyrosine kinase inhibitors [10], which act by interfering with ATP binding to the receptor.

Nimotuzumab is a humanized monoclonal antibody that targets the epidermal growth factor receptor. Nimotuzumab, also known as h-R3, is an anti-EGFr MAb developed at the Center of Molecular Immunology in Havana, Cuba. Originally isolated as a murine IgG2a anti-body, known as ior egf/r3, the MAb was humanized to reduce its immunogenicity and to slow clearance from the body by grafting the CDRs (complementarily-determining regions) of R3 to a human IgG1 gene [11]. In the process, the anti-body's variable fraction was further modified by recreating three specific murine amino acids (Ser 75, Thr 76, Thr 93) in order to preserve the new MAb's anti-EGFr activity [11].

Nimotuzumab is registered as a first-line treatment for head and neck cancer in combination with radiotherapy [12]. Nimotuzumab is currently being evaluated in several clinical trials: two Phase III trials as a first-line treatment for pediatric pontine and adult glioma, a Phase II/III trial as a treatment for pancreatic cancer, the phase II study in colorectal cancer reported in this release, phase I in tumors from epithelial origen. Some of those results are published already [13-17] and some of them are ongoing now.

The objective of this study was to characterize the pharmacokinetic profile of nimotuzumab when given in combination with doxorubicin and cyclophosphamide in patients treated with cumulative dose escalation regimen for each dose and each dose level administered, and to determine possible dose-dependent changes in the pharmacokinetics of nimotuzumab in patients treated with the multiple cumulative dose escalation regimen.

2. Materials and Methods

2.1 Patient Eligibility

Patients with histologically confirmed breast locally advanced-stage epithelial tumors that were not amenable to receive any further therapy and who had finished their last treatment at least 4 weeks before were included in the trial. Other selection criteria were a good performance status, normal hematological conditions, as well as normal hepatic and renal functions. The most important exclusion criteria consisted of previous treatments with murine anti-EGFr antibodies, pregnancy or lactation, serious chronic diseases, and active infections. All patients signed a written consent form before their inclusion in the clinical trial.

2.2 Study Design and Treatment Procedure

The study was designed as a clinical trial phase I, monocenter from scale up, clinical register number RPCE00000057 [18]. Twelve patients were included in four treatment cohorts, receiving multiple administrations of the monoclonal antibody. Three patients were enrolled in each of the following fixed dose levels: 50, 100, 200 and 400 mg/week. Nimotuzumab was administered weekly during 2.5 months by intravenous infusion of 0.5 hours. Subjects were closely monitored during the trial and finished the administration of nimotuzumab. The HAMA (human anti-mouse antibody) response was evaluated. Patients also received a combination of 60 mg/m^2 of Doxorubicin and 600 mg/m^2 of Cyclophosphamide in 4 cycles every 21 days intercalated with MAb. The trial was conducted under the principles outlined in the Declaration of Helsinki with the approval of the corresponding Ethics Review Committee for human subjects protection in clinical trials at the Hermanos Ameijeiras Hospital and the State's Center for Drug Quality Control (CECMED), the National Regulatory Agency.

2.3 Pharmacokinetics Assays

2.3.1 Drug Concentration Measurements

Serum samples were collected at week 1 and 10th immediately before IV infusion and 0, 1, 2, 4, 6, 7 days following the end of infusion, and before administration at 7th day and on every week before administration of nimotuzumab until week 9th.

Additional samples were collected after 10th administration before drug administration on 10th doses and 1, 6, 14, 20 and 26 days after the end of infusion. Samples were allowed to clot and then centrifuged. Serum was collected and stored at -20 °C. Serum concentrations of nimotuzumab were determined by a receptor-binding, ELISA (enzyme-linked immunosorbent assay), using the antigen HER 1, recombinant extracellular of EGFr domain to capture nimotuzumab from serum samples. Bound nimotuzumab was detected with sheep antihuman IgG gamma chain specific-alkaline phosphate (Sigma Chemical, A-3188, USA), and para-nitro-phenyl-phosphate diluted in diethanolamine was used as the substrate for color development to quantify serum nimotuzumab against a standard curve. Absorbance was read at 405 nm. The LLOQ (lower limit of quantification) of nimotuzumab in human serum was 7.5 ng/mL.

2.3.2 Pharmacokinetic Analysis

The individual concentration vs time profiles obtained after the first (day 1) and the tenth IV infusions (day 10) were analyzed by the NCA (non-compartmental analysis) using a combined linear/log linear trapezoidal rule approach. Pharmacokinetic calculations were performed using WinNonlin®, Pharsight® Co., 2006, ver. 5.3.

A time zero value was considered for extrapolation purposes. The linear trapezoidal rule was used up to peak level, after which the logarithmic trapezoidal rule was applied. Lambda *z* is a first-order rate constant associated with the terminal (log linear) segment of the curve. It was estimated by linear regression of the terminal data points. The largest adjusted regression was selected in order to estimate lambda *z*, with a caveat: if the adjustment did not improve, it was rather that within 0.0001 of the largest value the regression with larger number of points was used. For each patient in each dose level, metrics typically reported in pharmacokinetic studies were tabulated. Parameters extrapolated to infinity, using the moments of the curve, such as AUC (the area under the disposition curve), AUMC (the area under the first moment of the disposition curve) and MRT (mean residence time) were computed based on the last predicted level, where the predicted value is based on the linear regression performed to estimate terminal lambda first-order rate constant. Computing these parameters based on the last observed level was discouraged in order to avoid larger estimation errors.

The relationships between estimated pharmacokinetic parameters and administered weekly doses were assessed in order to determine the threshold level at which a dose proportionality is lacked.

2.4 Statistical Analysis

Descriptive statistical analyses (i.e., means, standard deviations) were performed to summarize the pharmacokinetic characteristics of participants in this study at each administered dose. Statistical comparison between the 1st and 10th administration in every dose level (i.e., 50, 100, 200 and 400 mg/week) was performed by a non-parametric Kruskal-Wallis test. All statistical analyses were performed using the SPSS software, version 15.0 (SPSS Inc., Chicago, IL, USA, 2006). Statistical significance was set at 5% ($P < 0.05$), with a 95% confidence interval.

2.5 Anti-Idiotypic Response

The anti-idiotypic response was evaluated pre-treatment, at day 7th and then weekly up to 2 months. The HAMA (human anti mouse antibody) response was considered to be positive when post-treatment value/pre-treatment ratio was higher than 2. It was determined by an ELISA (enzyme-linked immunosorbent assay), using the murine ior egf/r3 idiotype (CIMAB, D-0201). Briefly, 5 μg/mL of ior egf/r3 concentration was used as capture system overnight at 4 °C. Plates were washed and 1/400 dilutions of serum from nimotuzumab-treated patients were added. Plates were incubated for 1 hour at 37 °C and washed after adding

the antihuman IgG γ chain specific-alkaline phosphate conjugated and anti human IgM μ chain specific–alkaline phosphate conjugated (Sigma Chemical, A-3188 and A-9794, USA, respectively). After washing, then a chromogen solution (para-nitro-phenil-phosphate 1 mg/mL in diethanolamide buffer pH 9.8) was added and incubated by 30 min at room temperature. Plates were measured on an ELISA reader at 405 nm (Organon Teknika, Netherlans) [19].

3. Results and Analysis

3.1 Patient Characteristics

Twelve female patients, mean age 47 (30-63) years-old, with a histologically confirmed, advanced locally breast tumor were enrolled in the study. Participants were recruited from the medical facilities at the Hermanos Ameijeiras Hospital in La Habana, Cuba. Patient characteristics are detailed in Table 1.

3.2 Pharmacokinetics

The corresponding serum drug concentrations-time curves for the 1st and 10th administrations of nimotuzumab are depicted in Figs. 1 and 2, respectively, whereas, the means and standard deviations of the pharmacokinetic parameters for the first and tenth administration at each dose level are shown in Tables 2 and 3, respectively.

As expected, Fig. 3 shows a typical accumulative pattern after multiple doses of nimotuzumab given intravenously in each participant by intermittent short-term infusions. Besides, that non-proportional, greater than anticipated increments in the areas under the serum drug concentration-versus-time curves are observed across the dose range, which reveals a non-linear behaviour.

The mean $AUC_{0-\infty}$ values increased from 15601.75 to 71405.05 μg·h/mL after the 1st administrations of 50 and 400 mg/week, respectively, and from 20677.29 to 228797.09 μg·h/mL after the corresponding 10th administrations of the same dose levels, which indicate lack of dose proportionality (Fig. 4a).

The average value for the elimination half-lives ($t^{1}/_{2}$) of the humanized MAb in these patients was relatively long, and varies from 150.23 hours to 78.02 hours after the first administration of either 50 mg/week or 400 mg/week. Accordingly, the average drug CL (clearance) was relatively slow for all participants. These body weight-normalized CL values did not differ significantly along the dose range (i.e., oscillating from 0.05 mL/h·kg to 0.11 mL/h·kg), except for the 200 mg/week level that increases abruptly up to 0.43 mL/h·kg during the first administration. However, a decrease in the total clearance is observed after the 10th administration probably due to a saturation effect (Fig. 4b).

The average volume of distribution at steady-state (V^{ss}) was relatively small, suggesting a limited distribution out of the blood compartment or a significant binding to plasma/blood components. This parameter tends to increase after the 1st administration of the 200 mg/week dose level; whereas, these values fluctuated after the 10th administration (Fig. 4c).

When the pharmacokinetic parameters were compared across the different dose levels, there were found significant differences for $AUC_{0-\infty}$ of 0.019 and 0.033, C_{max} of 0.043 and 0.029 for 1st and 10th

Table 1 Demographic characteristics of the patients.

Variable	All patients (n = 12)
Gender	
Female	12 (100%)
Race	
White	8 (66.66%)
Black	4 (33.33%)
Age (years)	
Median	49
Average (Range)	47 (30-63)
Overall condition as per ECOG	Less 2
Median (Range)	12 (100%)
Histology	
Ductal carcinoma	11 (91.66%)
Lobular carcinoma	1 (8.33%)
Degree of differentiation	
Intermediate malignancy grade	2 (16.66%)
High malignancy grade	8 (66.66%)
Low malignancy grade	2 (16.66%)

administration respectively, and CL during the 1st administration (0.031), but not for the 10th administration indicating the saturation levels of the nimotuzumab followed doses multiple regimen (Tables 2, 3 and Fig. 4).

Table 4 presents the estimated average drug concentrations at steady state ($C^{ss}_{average}$) and the peak and trough steady-state concentrations of nimotuzumab for patients in the four different dose levels. The C^{ss} average values increase disproportionately to the dose levels. Indeed, it is observed that at the dose of 200 mg/week the C^{ss} average is almost three times that at 100 mg/week, which could indicate that a dose-dependent non-linearity process is involved in the elimination of nimotuzumab.

3.3 Anti-Idiotypic Response

After the evaluation of the human response against the murine portion of the MAb (using an ELISA test), it was verified that the optical density values were in all cases very similar to the pre-treatment values for the IgM and IgG responses (Fig. 5).

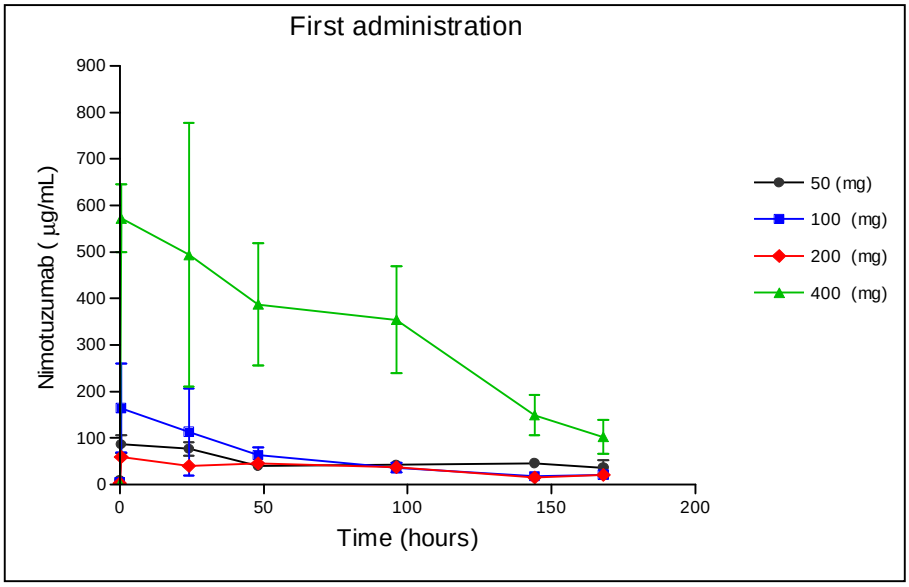


Fig. 1 Nimotuzumab mean serum concentration–time profiles in first administration for four doses level.

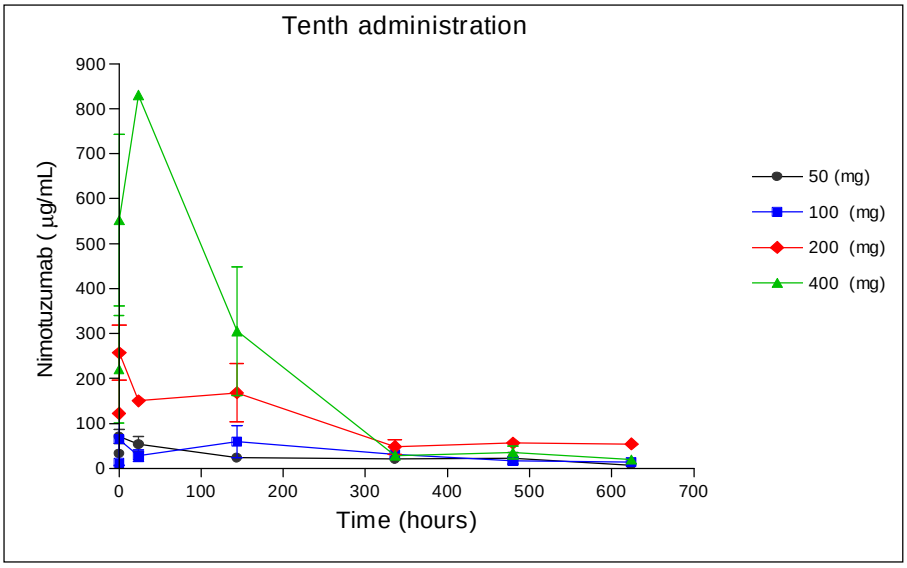


Fig. 2 Nimotuzumab mean serum concentrations–time profiles in tenth administration for the 50, 100, 200 and 400 mg/week doses.

Table 2 Nimotuzumab pharmacokinetic parameters for the 1st administration. Non-Compartmental Analysis.

Dose (mg)	No. of patients	$AUC_{0-\infty}$ (µg·h/mL)	C_{max} (µg/mL)	t ½ (h)	CL/kg (mL/h·kg)	Vss/kg (mL/kg)
50	3	15601.65 ± 5152.58	79.96 ± 22.16	150.23 ± 69.91	0.05 ± 0.01	11.24 ± 2.6
100	3	11186.87 ± 2105.47	169.67 ± 99.52	63.72 ± 46.32	0.13 ± 0.03	11.25 ± 9.4
200	3	7879.41 ± 448.75	59.59 ± 4.32	88.66 ± 9.39	0.43 ± 0.04	54.30 ± 8.4
400	3	71405.05 ± 37116.59	499.53 ± 27.58	78.02 ± 8.78	0.11 ± 0.08	11.93 ± 7.5
(*P*)		0.019*	0.043*	0.154	0.031*	0.082

Values are mean ± SD. $AUC_{0-\infty}$, area under plasma drug concentration-time curve from zero to infinity; C_{max}, maximum level of concentration; t ½, half-life; CL/kg, clearance corrected per kg of weight; Vss/kg, volume of distribution at steady-state corrected per kg of weight. *P*: Statistical significance < 0.05; IC_{95}: Kruskal-Wallis test.

Table 3 Nimotuzumab pharmacokinetic parameters 10th administration. Non-Compartmental Analysis.

Dose (mg)	No. of patients	$AUC_{0-\infty}$ (h·µg/ mL)	C_{max} (µg/mL)	t ½ (h)	CL/kg (mL/h·kg)	Vss/kg (mL/kg)
50	3	20677.29± 6881.28	73.98 ± 11.72	274.20 ± 66.59	0.04 ± 0.02	16.00 ± 4.94
100	3	25489.90 ± 10656.01	64.58 ± 57.06	355.62 ± 145.01	0.06 ± 0.03	33.07 ± 22.7
200	3	71765.77 ± 35480.68	257.63 ± 106.9	218.62 ± 5.08	0.05 ± 0.02	17.91 ± 8.38
400	3	228797.09 ± 228232.98	582.4 ± 325.31	105.76 ± 19.76	0.08 ± 0.07	11.47 ± 10.7
(*P*)		0.033*	0.029*	0.082	0.705	0.459

Values are mean ± SD. $AUC_{0-\infty}$, area under plasma drug concentration-time curve from zero to infinity; C_{max}, maximum level of concentration; t ½, half-life; CL/kg, clearance corrected per kg of weight; V^{ss}/kg, volume of distribution at steady-state corrected per kg of weight. *P*: Statistical significance < 0.05, IC_{95}: Kruskal-Wallis test.

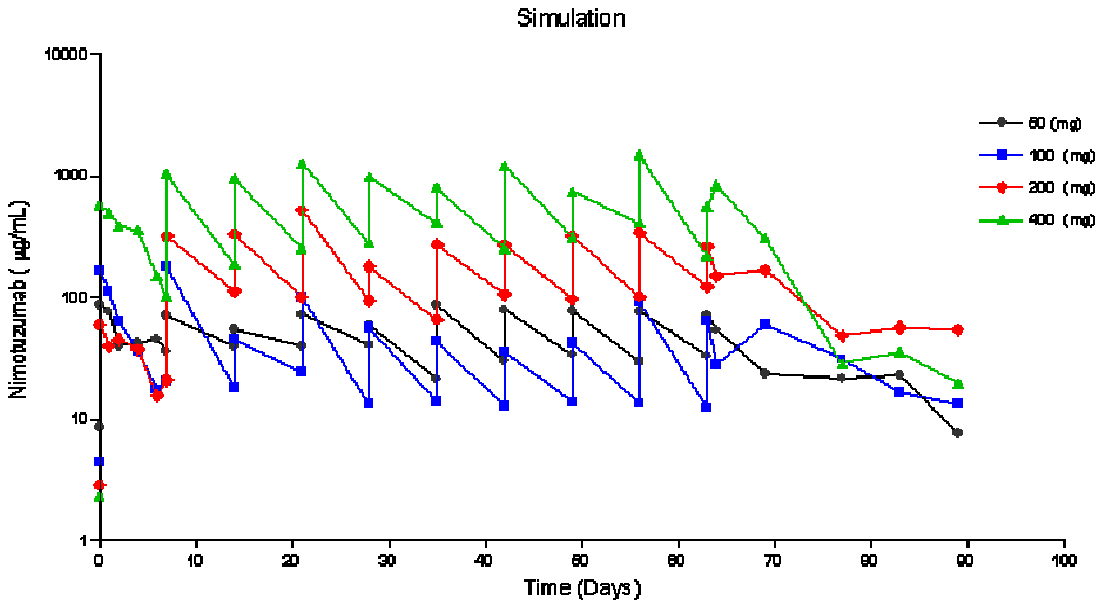
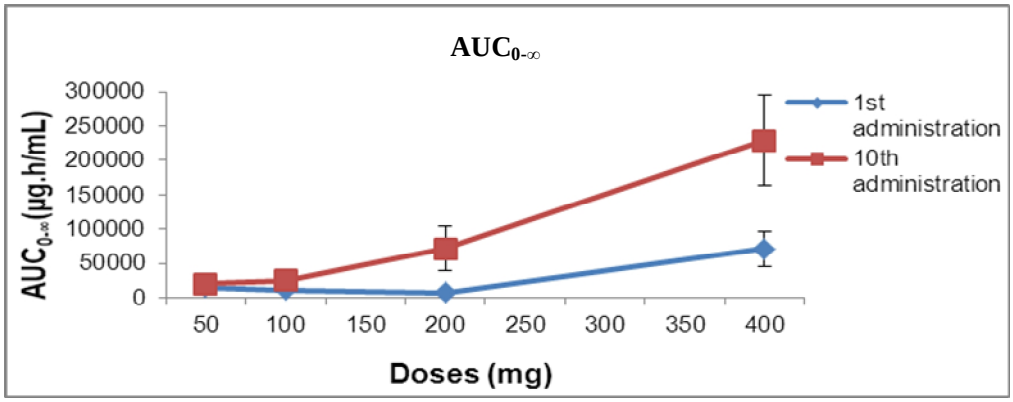
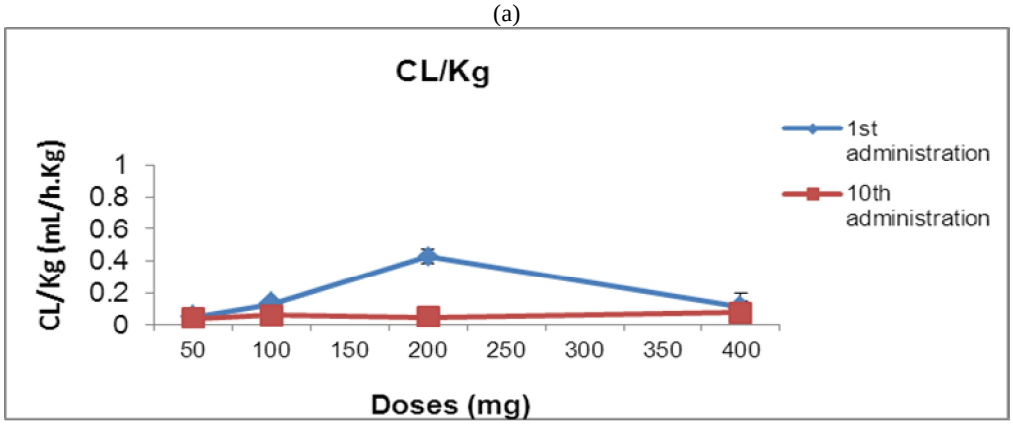


Fig. 3 Nimotuzumab concentration-time data in multiple administration regime. Graph shows the observed nimotuzumab concentrations represented as symbols and color lines with black rhombus, blue square, red rhombus and green triangle for the 50, 100, 200 and 400 mg/week doses, respectively.

Once the post-treatment/pre-treatment ratio of ≥ 2 was established as the cohort value to consider if a patient would have a positive anti-idiotypical response, it was confirmed that none of the patients treated had a higher value. Therefore, it can be considered that with 10 doses of the MAb, even after the single dose was increased to 400 mg/week and the total dose being 4000 mg, the patients did not develop a response against the murine portion of the nimotuzumab, which shows low immunogenicity of this MAb due to its humanized characteristics. See Fig. 5 for anti-idiotypic response, anti-IgG and anti-IgM graphs.



(a)



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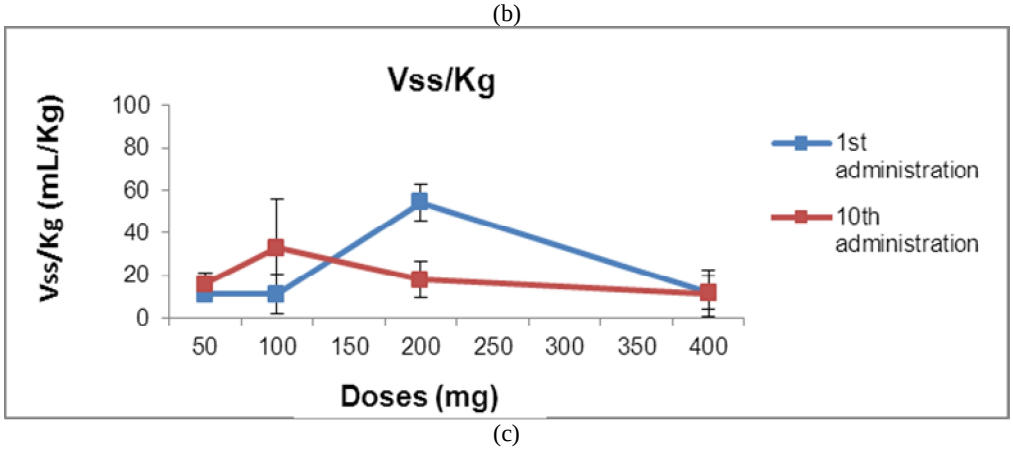

(c)

Fig. 4 a, Area under the serum concentration time curve from time zero to infinity ($AUC_{0-\infty}$) as a function of the nimotuzumab dose from 50 to 400 mg/week (n = 12); b, CL/kg (Clearance corrected per kg of weight) as a function of the nimotuzumab dose from 50 to 400 mg/week (n = 12); c, Vss/kg (volume of distribution at steady-state corrected per kg of weight) as a function of the nimotuzumab dose from 50 to 400 mg/week (n = 12).

Table 4 Estimates average concentration in the steady state and maximal and minimal concentration in the steady state of Nimotuzumab.

Dose (mg)	No. of patients	$C^{ss}_{average}$	C^{ss}_{max}	C^{ss}_{min}
50	3	122.53 ± 38.27	149.39 ± 44.0	98.76 ± 33.02
100	3	160.57 ± 63.51	196.26 ± 93.75	129.93 ± 41.83
200	3	465.6 ± 290.37	592.53 ± 369.52	357.89 ± 223.19
400	3	864.72 ± 550.40	1379.27 ± 869.70	498.67 ± 333.47

Values are mean ± SD. $C^{ss}_{average}$: estimates average concentration in the steady state; C^{ss}_{max}: maximum concentration in the steady state; C^{ss}_{min}: minimum concentration in the steady state.

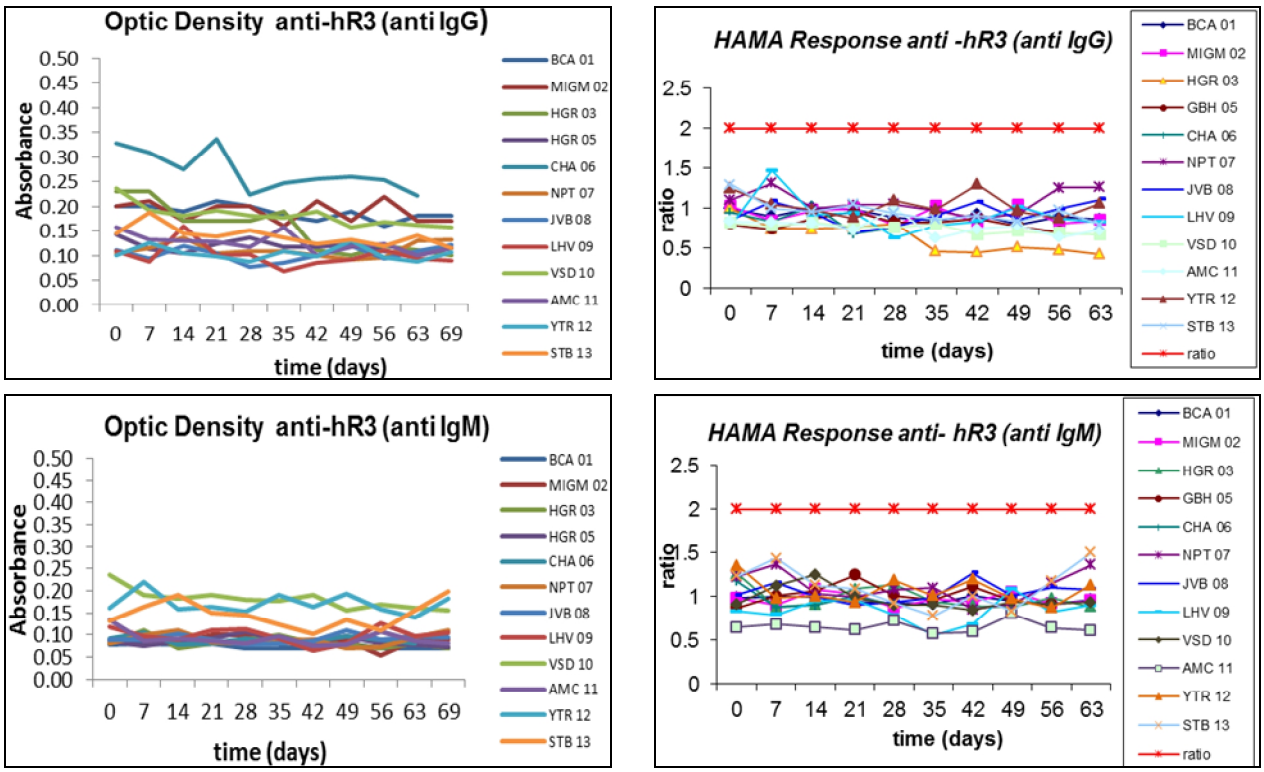

Fig. 5 Anti-idiotypic response in treated patients (n = 12).

4. Discussion

Despite the advances in surgery, radiation and chemotherapy, advanced epithelial-derived cancer largely represent an unsolved problem. Today, biologic therapy emerges as the fourth modality for cancer management, being a very attractive option taking into consideration its specificity and low toxicity [20]. Passive immunotherapy against solid tumors with naked antibodies has recently demonstrated efficacy in the clinical setting [20].

EGFr is a very attractive target for immunotherapy since EGFr driven autocrine growth pathway has been implicated in the development and progression of the majority of human epithelial cancers [21].

Breast cancer shows an increase in the EGFr expression, and it has been reported that 14-91% of tumours over-express this receptor [2]. The action of nimotuzumab plus AC chemotherapy was evaluated in this study. Given the fact that nimotuzumab competitively inhibits the binding of the EGF ligand to the extracellular domain of the HER-1 protein, leading to a further inhibition of the homodimerization or heterodimerization of this receptor and subsequent autophosphorylation of its tyrosine residues, the activation of the different ligand-induced signal transduction pathways (Ras-Raf-MEK-MAPKS cascade; PI3K) will be inhibited as well [10]. The aim was to assess the pharmacokinetic of nimotuzumab in patients with locally advanced breast cancer who are receiving neoadyuvant therapy combined with the AC chemotherapy regimen. Moreover, AC acts directly on the nucleus of the cells that are in turnover phase and therefore the replication of the DNA would be inhibited and the combination of both therapeutic agents potentiates the antitumour effect (Fig. 3).

Cetuximab (chimeric monoclonal anti-EGFR antibody) combined with antineoplastic agents showed a synergistic effect and an increase in the anti-tumour efficacy in metastatic colorectal cancer and head and neck tumors [22]. It is suggested that the MAb Erbitux presents synergistic activity with many antineoplastic agents such as cisplatin, doxorubicin,

paclitaxel and irinotecan [22].

Trastuzumab, a humanized MAb that recognizes the HER-2 receptor (a member of the EGFr family) was licensed in 1997 for the treatment of metastatic breast cancer combined with paclitaxel [23]. Different clinical studies have led to an extension of this therapy to the earlier stages of breast cancer, based on the fact that this molecule blocks HER2, which is part of the EGFr group [23].

Nimotuzumab was combined with an anthracycline based chemotherapy (i.e., doxorubicin-cyclophosphamide) in the phase I clinical study. This new therapeutic regimen for nimotuzumab included an increase in the number of administrations up to ten doses. The first dose of this MAb was administered before starting chemotherapy in order to induce an effect of nimotuzumab on a HER 1 over-expressing tumour without any interference of the cytostatic therapy as well as to assess the pharmacokinetics of nimotuzumab in this type of patient and therapy.

To our knowledge, this is the first time a pharmacokinetic report on Nimotuzumab combined with chemotherapy after multiple dosing in breast cancer patients is written. The pharmacokinetic analyses for nimotuzumab showed a lack of dose proportionality within the dose range of 50-400 mg weekly, as suggested by a disproportional increase of $AUC_{0-\infty}$ over the entire dose range and dose-dependent clearance and apparent distribution volumes. This is typically the non-linear pharmacokinetic behavior that has been early reported for other monoclonal antibodies in humans [13, 22, 23].

Clearance variations observed over the entire dose range and the resulting dose-dependency pattern in this study was opposed to some previous reports for this (i.e., I^{125}-labelled nimotuzumab) and other MAbs [13, 22, 23]. However, the results of the current study correlate well with an early report from a pharmacokinetic study of nimotuzumab in patients with locally advanced or metastatic pancreatic cancer that was conducted in Germany [24].

Although no statistically significant differences are found between clearance values of this MAb after the 10th administration (Table 3), results after the 1st administration (Table 2) showed a slight but significant variation of the MAb clearance in these patients ($P = 0.03$), with total clearance rising steadily up to the dose of 200 mg/week and then a sudden decrease is observed at the 400 mg/week dose. Since there is a turning point in the total systemic clearance of nimotuzumab at the interval of 200 mg/week to 400 mg/week, we consider this dose range as one representing a saturation region for its biological targets or reservoirs in the body and thus a potential optimal dose level. An explanation for the clearance variations observed in the dose range (i.e., 50-200 mg/week) tested in this study cohort could be found in the additive nature of this parameter: CL Total = specific CL + non-specific CL [25].

We speculate that the total systemic clearance reduction at the very high dose is probably a consequence of the saturation of the membrane-bound EGFr-mediated elimination pathway (i.e., a specific clearance routes with limited capacity such as receptor-mediated endocytosis). The impact of the antigen/target on the non-linear pharmacokinetics of MAbs is generally characterized by faster clearance rates at a lower dose range; whereas, the clearance decreases at the highest dose of the antibody due to a saturation of the specific antigen/target-mediated clearance process. However, non-specific processes (e.g., RES (reticule-endothelial system)-mediated events) are likely favoured at increasing doses [25] and, therefore, they might account for most of the observed changes in clearance at the studied dose range.

On the other hand, the systemic clearance of MAbs in cancer patients could be modified by several factors including soluble antigen in circulation and immunogenicity [25]. For instance, previous reports from a phase I clinical trial study revealed an increased clearance of trastuzumab, which was associated with high levels of shed antigen [23]. In another study of

nimotuzumab given to 12 patients with advanced epithelial-derived cancer, Crombet et al. observed that 3 patients with ductal infiltrating breast carcinomas had positive serum elevation of shedding EGFr [13]. Since we have patients with advanced breast cancer in our study cohort, we believe that a fraction of the nimotuzumab in the bloodstream is bound to these circulating antigens, and that nonspecific RES clearance process [25] is also contributing to the increasing clearance value observed across the dose ranges from (50 mg/week to 200 mg/week). In fact, it seems to be that EGFr shedding correlated with nimotuzumab clearance after the 1st administration.

Increased serum ECD (Extracellular Domain) levels of EGFr have been early reported in patients with cancers that are known to overexpress Her 1 or Her 2 protein [23, 26, 27]. In a study with patients suffering from metastatic breast cancer who were receiving 2 mg/kg of trastuzumab, a patient with high circulating shed antigens had a similar reduction of serum concentrations to that observed in our study after giving the dose of 200 mg/week during the 1st administration [23]. This report suggests that high circulating antigens will decrease the elimination half-life and the trough serum concentrations of humanized MAb against HER2 product [23]. Besides, high serum concentrations of HER 2 ECD have been correlated with higher relapse rates, and elevated pretreatment levels of HER2 ECD have also been associated with poor clinical response to hormone therapy and chemotherapy in metastatic breast cancer patients [27]. Therefore, monitoring of circulating shed antigen level is considered to be essential in trastuzumab therapy [27].

Notably, these shed EGFr molecules do not seem to significantly interfere with the clearance of nimotuzumab after the 10th administration cycle. It might be due to a depletion of circulating antigens after multiple administrations of the MAb.

The human anti-murine antibody response does not alter the clearance of nimotuzumab. The lack of anti-idiotypic response in this study emphasizes the relatively non-immunogenicity of this humanized MAb following repeated administrations of nimotuzumab the doses. In previous studies by Crombet et al., the anti-idiotypic antibodies were not detected in any patient after IV infusions of nimotuzumab at several dose levels, with measurements performed up to 6 months after treatment [13].

A limitation of our study is the relatively small sample size to perform the corresponding statistical analyses with power enough to draw valid conclusions. Accordingly, results and recommendations should be observed with cautions.

5. Conclusion

Nimotuzumab showed a non-linear dose-dependent pharmacokinetics. No interactions between the administration of nimotuzumab and chemotherapy (doxorubicin plus cyclophosphamide) were observed at the studied dose levels. No anti-idiotypic response to nimotuzumab was found in the patients enrolled in this study. Monitoring of circulating shed EGFr level must be considered in nimotuzumab therapy for breast cancer. Based on our findings, we preliminarily recommend the 200 mg/week to 400 mg/week infusion dose range as the OBD (Optimal Biological Dose) range to be proposed for further human studies of this MAb.

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