PERGAMON

European Journal of Cancer 38 (2002) 1072–1080

European Journal of Cancer

www.ejconline.com

Phase I and pharmacokinetic study of BIBX 1382 BS, an epidermal growth factor receptor (EGFR) inhibitor, given in a continuous daily oral administration

Ch. Dittrich[a,*], G. Greim[b], M. Borner[c], K. Weigang-Köhler[b], H. Huisman[d], A. Amelsberg[e], A. Ehret[e], J. Wanders[d], A. Hanauske[f], P. Fumoleau[g] for the EORTC Early Clinical Studies Group (ECSG)

[a]*Ludwig Boltzmann-Institute for Applied Cancer Research, (LBI-ACR VIEnna), KFJ-Spital, 3rd Medical Department, Oncology, Kundratstraße 3, A-1100 Vienna, Austria*
[b]*5th Medical Clinic, Klinikum Nürnberg Nord, Germany*
[c]*Department of Oncology, Inselspital, Bern, Switzerland*
[d]*NDDO-Oncology, Amsterdam, The Netherlands*
[e]*Boehringer Ingelheim, Biberach, Germany*
[f]*Technical University, Munich, Germany*
[g]*Centre René Gauducheau, Nantes, France*

Received 22 November 2001; accepted 23 November 2001

Abstract

The pyrimido-pyrimidine BIBX 1382 BS inhibits the intracellular tyrosine kinase domain of the epidermal growth factor receptor (EGFR), thus specifically reverting the aberrant enzymatic activity from overexpressed and constitutively activated EGFR. A phase I and pharmacokinetic study of this new specific molecule was carried out. After initially performing an accelerated titration design from the first toxicities onwards, a modified Fibonacci scheme was used to escalate the daily oral dose. The following dosages and cycles (defined as treatment during 28 days) were applied: 25 mg: 6; 50 mg: 3; 100 mg: 6; 200 mg: 7; 150 mg: 3. Over a 10 months accrual phase, 11 patients (pts) (7 females, 4 males) with a median age of 63 years (range 50–73 years), World Health Organization Performance Status (WHO PS) 0:5 pts, 1:6 pts and miscellaneous solid tumours were entered. The median number of cycles applied per pt was 2 (range 1–7). Reversible, dose-dependent increase of liver enzymes (maximal Common Toxicity Criteria (CTC) grades: gamma-glutamyl transferase (GGT): 4, aspartate aminotransferase (GOT): 3, alanine aminotransferase (GPT): 3, alkaline phosphatase (AP): 3, bilirubin: 3) occurred. Oral medication yielded plasma levels far below those expected to be efficacious. In conclusion, target plasma levels could not be reached via the oral route at a reasonable dosage. Meanwhile, a preclinically unknown metabolite was identified from the urine of one patient. Subsequently, this metabolite was found to be abundant in patient plasma. The metabolite was demonstrated to be pharmacologically inactive. Due to a dose-limiting increase of liver enzymes, low bioavailability of BIBX 1382 BS and the detection of a pharmacologically inactive metabolite, this trial was discontinued.

Keywords: BIBX 1382 BS, Epidermal growth factor receptor (EGFR)-inhibitor; Pyrimido-pyrimidine; Tyrosine kinase inhibitor; Phase I; Pharmacokinetics

1. Introduction

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase, i.e. following stimulation by its peptide ligands (EGF, transforming growth factor (TGF)-alpha and others), it phosphorylates protein substrates on tyrosine and thereby activates intracellular signal transduction cascades that eventually induce cell proliferation, impair cell differentiation and, in some instances, enhance cell survival [1–3], all phenomena implicated in neoplastic transformation. EGFR inhibitors are expected to display a more selective toxicity for tumour cells than classical cytotoxic chemotherapeutics that indiscriminately affect all proliferating cells. It is

* Corresponding author. Tel.: +43-1-60191-2303/2301; fax: +43-1-60191-2329.
E-mail address: christian.dittrich@kfj.magwien.gv.at (C. Dittrich).

0959-8049/02/$ - see front matter
PII: S0959-8049(02)00020-5

believed that tumour cells rely on EGFR signalling to a greater extent than normal cells do. While often only EGFR is constitutively activated in the tumour cells, the normal cells co-express a number of receptor tyrosine kinases at similar levels as EGFR and these other molecules can probably replace EGFR functionally [2]. Therefore, it is expected that tumour cells will be hit harder by EGFR inhibitors than normal cells.

EGFR inhibitors are not expected to kill tumour cells directly or to cause a rapid disappearance of tumours. Rather, they inhibit tumour cell proliferation and are, therefore, expected to restrict further tumour growth. As a consequence, it is likely that EGFR inhibitors have to be administered over long time periods. In such a setting, administration via the oral route is a must. Moreover, it is essential for a successful therapy that EGFR signalling in tumour cells is inhibited continuously, because brief pulses of EGFR signals, if they occur daily, could suffice to maintain tumour cell proliferation.

Anti-EGFR antibodies act as receptor antagonists, which bind to the extracellular portion of EGFR [4] and, thus, prevent the binding of activating ligand, whereas small molecules such as BIBX 1382 BS, the pyrimido-pyrimidine N8-(3-chloro-4-fluorophenyl)-N2-(1-methyl-4-piperidinyl)-pyrimido[5,4-d]pyrimidine-2,8-diamine, pyrimido-pyrimidine, inhibit the intracellular tyrosine kinase domain of the EGFR. Inhibitors of the EGFR tyrosine kinase are expected to specifically reverse the aberrant enzymatic activity of EGFR occurring in tumour cells [2,5].

The antitumour activity of BIBX 1382 BS has been evaluated in murine tumour models and in human tumour lines both *in vitro* and *in vivo*. The antitumour efficacy of BIBX 1382 BS was tested in the mouse tumour model F/Lauto. BIBX 1382 BS inhibited EGFR-dependent proliferation of this test cell line (a recombinant derivative of mouse myeloid FDC-P1 cells expressing human EGFR) by 50% at 10 nM compound concentration. Half-maximal inhibition of the proliferation of similar cells driven by HER-2 (the closest relative of EGFR) occurred at 1000 nM concentration, while cell proliferation relying on other receptor tyrosine kinases, non-tyrosine kinase growth factor receptors or signal transducers was not affected by compound concentrations of 3500 nM or higher, demonstrating potent and selective inhibition of EGFR signalling by BIBX 1382 BS and its metabolite BIBX 1330, as well as penetration of the compound through the cell membranes ([6]; Metz and data not shown); BIBX 1382 BS caused full growth suppression of established subcutaneous (s.c.) A431 tumours during a 3-week treatment period with dosages decreasing down to 10 mg/kg/day. Regrowth after termination of therapy indicated the necessity of continuous treatment to maintain permanent tumour suppression.

Various degrees of antitumour effect of BIBX 1382 BS ranging from a reduced growth rate to complete growth suppression could be detected at the human EGFR-expressing head and neck squamous cell carcinoma cell line-derived s.c. nude mouse tumour models FaDu HN5, HN15 and HNX-OE. Only in the EGFR-expressing KB cell line was no antitumour effect found (data not shown).

BIBX 1382 BS and its metabolite BIBX 1330 have been found to bind to alpha-1 acid glycoprotein (AGP) *in vitro* (data not shown). The acute phase serum protein AGP reduces the potency of BIBX 1382 BS and BIBX 1330 for EGFR inhibition in a dose-dependent manner in the physiologically relevant AGP concentration range of between 0.5 and 5 mg/ml. Since AGP plasma levels in human cancer patients (0.3–5 mg/ml) are approximately 10-fold higher than mouse AGP plasma levels, the consequences of plasma AGP for pharmacokinetics and antitumour efficacy cannot be easily tested in mice [7].

In human liver microsomes, it has been found that BIBX 1382 BS is mainly metabolised by CYP2D6, an isoenzyme of the cytochrome P450 system. This enzyme is subject to genetic polymorphism, resulting in poor and extensive metabolisers.

Preclinical pharmacological investigations indicated that the drug is comparably well absorbed after oral administration in rats and mice; absolute bioavailability ranges between 50 and 100%, respectively, despite low solubility at a pH value of >6. Preliminary metabolic studies indicated that the drug is demethylated in the mouse and rat (to BIBX 1330). The volume of distribution with 10–20 l/kg is high. BIBX 1382 BS is cleared from plasma at a rate of 55 ml min^{-1} kg with a half-life of approximately 4–6 h for the lower doses and extended to approximately 10 h for the high-dose groups in the two species. The area under the curve (AUC) showed a less than dose-dependent increase, indicating a non-linearity in the disposition of BIBX 1382 BS.

Preclinical toxicology studies were performed in mice and rats covering various modes, starting from single intravenous (i.v.) injections, covering long-term oral gavage up to 3 months revealing the most pronounced treatment-related changes in a dose-dependent manner.

In mice, BIBX 1382 BS caused toxicity of the skin, microscopically defined as dermatitis/hair folliculitis, toxicities of the heart in form of arteritis/endocarditis, haematotoxicity with decreased red blood cell count, hepatotoxicity with increased activity of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT), as well as an increase in the total number of white blood cells, lymphocytes and platelets. In rats, hepatotoxicity was more pronounced yielding microscopically detectable necrosis and inflammation. In addition, renal damage and treatment-related lesions including lungs, thymus, stomach, pancreas and ovaries were seen. No Observed Adverse Effect Levels (NOAEL) for long-term oral application of BIBX

1382 BS for mice and rats were 10 and 5 mg/kg/day, respectively.

The above characterisations, including the reversibility of the toxic effects, made the substance an attractive candidate for further clinical development.

2. Patients and methods

2.1. Patients

Adult patients, i.e. aged 18 years or more, with histologically- or cytologically-confirmed advanced solid tumours not amenable to established forms of treatment were candidates for this study. Eligibility criteria corresponded to the standards for phase I. All patients were required to give written informed consent. Before commencing the study, ethical committee approval of the respective regional ethical committee was obtained. The study was conducted according to the Declaration of Helsinki and the International Conference on Harmonisation (ICH) of Good Clinical Practice (GCP) guidelines.

2.2. Methods

2.2.1. Study design

This was an open label, non-randomised study. The study was meant to consist of two stages:

- a dose escalation phase, until the maximum tolerated dose (MTD) had been reached;
- a dose selection phase intended once the MTD had been reached.

The detailed study objectives for this trial were defined as follows:

a. to determine the MTD of BIBX 1382 BS, when administered once daily continuously as an oral formulation;
b. to determine the qualitative and quantitative toxic effects of BIBX 1382 BS and to study the predictability, duration, intensity, onset, reversibility and dose-relationship of the toxic side-effects;
c. to propose a safe dose (i.e. near MTD) for phase II evaluation;
d. to study the pharmacokinetics of BIBX 1382 BS in man at different dose levels;
e. to document any possible antitumour activity;
f. to select the optimal dose for phase II studies, if possible.

2.2.2. Dose escalation

Starting dose was 25 mg total dose per day corresponding to one half of the NOAEL in mice and rats (being approximately 30 mg/m^2 in both species) (Table 1). Toxicity was graded according to the National Cancer Institute (NCI)-Common Toxicity Criteria (CTC), version 2.0. The dose was escalated according to an accelerated titration design for phase I studies with single patient cohorts and double-dose step escalation until CTC grade 1 non-haematological (except alopecia, anaemia, untreated nausea/vomiting) or CTC grade 2 haematological toxicity was reached. From then on, 3 patients per dose level were to be treated. Once toxicity was observed, doses were to be escalated according to the modified Fibonacci Scheme.

Dose limiting toxicity (DLT) was defined as:

1. grade 3 non-haematological toxicity (except inadequately treated nausea/vomiting);
2. grade 4 haematological toxicity
3. interruption of treatment due to toxicity for >1 week;
4. maximum number of capsules which can be taken by the patient.

MTD was defined as: DLT in $\geqslant 2$ out of 6 patients.

Once the MTD had been reached, i.e. in the dose-selection stage, 10 patients having tumour types which are likely to express EGFR were to be entered at three different (below MTD) dose levels. The aim of this part of the study was to get at least an indication of the biological activity at the different dose levels and to assist in the selection of a dose for early phase II studies.

2.2.3. Pretreatment and follow-up studies

Before study entry, a medical history, complete physical examination, full blood count, serum chemistry, urinalysis and an electrocardiogram were performed.

Furthermore, a blood sample for *CYP2D6* genotyping was taken for each patient and the plasma concentrations of AGP was determined on day 1 prior to the first dose of medication being administered.

All patients who had received a single application of the drug in whatever preparation (solution for i.v. injection or tablet) were assessed with regard to toxicity.

In patients with measurable or evaluable disease, tumour response was assessed and classified according

Table 1
Dose escalation of BIBX 1382 BS

Dose level (total dose) (mg)	Patients (n)[a]	cycles (n)[b]
25	1	6
50	1	2
100	1	2
200	5	7
150	3	3
5 dose levels	11 patients	20 cycles

[a] Per dose level allocated.
[b] Of patients allocated to the dose levels.

to the World Health Organization (WHO) criteria [8] every two cycles, i.e. 8 weeks. When a response was observed, this had to be confirmed 4 weeks later. When progression was observed after 3 weeks, the patient was considered to be an 'early progression'. Patients removed from the study at earlier times for whatever reason were considered non-evaluable for response. Responding patients or patients with stable disease continued treatment until tumour progression or unacceptable toxicity, whichever occurred first, while patients with progressive disease were taken off study.

2.2.4. Drug administration

BIBX 1382 BS (raw material) was supplied by Boehringer-Ingelheim (Biberach, Germany). Using this material, an i.v. formulation and oral (tablet) formulation were produced at The Netherlands Cancer Institute/Slotervaart Hospital, Department of Pharmacy and Pharmacology, Amsterdam, The Netherlands. The i.v. formulation is a yellow powder to be diluted with water for injection (WFI) to a concentration of 20 mg/ml and mixed by using a vortex to provide a clear solution. The necessary amount of drug had to be diluted with 20 ml of WFI to be injected through a running i.v. infusion. Tablets containing 25, 100 or 200 mg/tablet had to be taken after an overnight fast, at least 1 h before a meal.

2.2.5. Pharmacokinetics

2.2.5.1. Sample collection and storage. Pharmacokinetic studies were performed in all patients after a single i.v. (day 1) and single oral administration (day 8) of BIBX 1382 BS. Blood samples were collected in ethylene diamine tetraacetic acid (EDTA)-containing tubes. After single i.v. administration (day 1), blood was taken at the following time-points: before administration, 30 minutes after the start of administration, then just before and after the end of administration and at the following time-points after the end of administration: 10, 30, 90 min, 4, 6, 10, 20–24, 28–32, 44–48 and 68–72 h after the end of administration. The time-points for the single oral administration (day 8) were: just prior to the administration and at the following time-points after the administration: 1, 1.5, 2, 2.5, 3, 4, 6, 10, 20–24, 28–32, 44–48, 68–72 h. Samples for repeated daily oral dosing were collected on day 1 and on days 7, 14, 21 and 28 after the start. The samples were stored at -20° C.

Urine samples were collected on the day of the i.v. and the oral administration course. One sample was taken prior to the administration. The total urine output was collected on two 24-h intervals, i.e. 0–24 and 24–48 h. An additional spot sample was collected roughly at the sample time of the 68–72 h plasma sample.

The collection container(s) were kept in a refrigerator. The urine collected during this time period was to be thoroughly mixed, the total volume recorded on the collection form and two duplicate samples of 10 ml were to be transferred to a polypropylene tube, tagged and stored at $-20\ ^{\circ}$C.

2.2.5.2. Bioanalysis. Analytical chemistry was performed at the Chemistry Department of NOTOX B.V. (Hambakenwetering 7. 's-Hertogenbosch, The Netherlands). The levels of BIBX 1382 BS and BIBX 1330 BS were determined in the plasma and urine samples. The methods were set-up and completely validated by NOTOX B.V. Both of the methods in the plasma and urine were based on high performance liquid chromatography (HPLC) with mass spectrometric detection and were suitable for measurement of BIBX 1382 BS and BIBX 1330 BS in the plasma ranging from 0.2 to 1000 ng/ml and in the urine ranging from 0.5 to 1000 ng/ml. As part of each set of samples, system performance (expressed as Coefficient of Variation (CV) (%)), linearity of response (expressed as correlation coefficient 'r^2' of weighed linear regression based on least square method), accuracy (Relative Error (RE)(%)) and influence of dilution, were evaluated against the acceptance criteria ($CV < 15\%$, $r^2 > 0.98$, $RE < \pm 15\%$ for calibration standards (for the lowest alibration standard $< \pm 20\%$) and $< \pm 20\%$ for the quality control samples (QCs).

All analytical batches were in agreement with the acceptance criteria, except for batch 4 for BIBX 1330 BS. However, due to the measured low concentrations for BIBX 1330 BS in this batch, reanalysis was not performed.

It was concluded that sample results were of sufficient integrity and quality.

2.2.5.3. Pharmacokinetic analysis. Based on the individual plasma concentration–time data, the relevant pharmacokinetic parameters for i.v. and per oral administration defined below were determined after each dose, using the computer program TopFit 2.0 [9].

C_{end}: plasma concentration at the end of the infusion;

C_{max}: maximal plasma concentration, determined by visual inspection of the data;

t_{max}: time to reach the peak plasma concentration, determined by visual inspection of the data;

AUC_{last}: AUC calculation using the last measurable concentration (C_{last});

$AUC_{0-\infty}$ area under the curve after a single dose, calculated as $AUC_{048h} + C_{48h}/\beta$, where C_{48h} is the BIBX 1382 BS concentration measured in the sample taken 48 h after the start of administration. If the data-set did not allow calculation to 48 h, then AUC_{last} was calculated and the last measurable concentration (C_{last}) was used. Extrapolations of more than 15% of the total AUC are reported as approximations;

$t_{1/2}$: terminal half-life, defined as Ln(2)/β;
MRT: mean residence time, calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$, where AUMC is the area under the first moment curve.

2.2.5.4. Statistical analysis. Plasma concentration–time data were subjected to an exploratory graphical analysis including various transformations in order to get a general overview. A common descriptive statistical and graphical analysis of the noncompartmental pharmacokinetic parameters (C_{end}, C_{max}, t_{max}, AUC_{last}, $AUC_{0-\infty}$, $t_{1/2}$, MRT) was performed using the computer program Nonmem [10].

Non-linear mixed-effect modelling was applied to assess the effect of dose and other potential covariates on plasma clearance and volume of distribution of the compound and to estimate inter- and intra-individual variabilities of these parameters.

3. Results

3.1. Patient characteristics and dose escalation

The entire study population comprised 11 patients, entered from January until November 1999 by three different centres. Overall, these 11 patients received a total of 25 cycles of treatment with BIBX 1382 BS. The median number of cycles per patient was 2, with a range of 1–7 cycles.

The mean age of the 11 patients entered into the study was 61 years (median 63 years), ranging from 50–73 years. A total of 4 males and 7 females were recruited. The performance status was 0–1 (WHO-scale): 5 patients had an initial performance status of 0, 6 patients had an initial performance status of 1. Prior therapeutic measures consisted of radiotherapy in 4 patients, systemic therapy in 8 patients, surgery in 9 patients.

3.2. Toxicities related to BIBX 1382 BS

3.2.1. Haematological toxicity

There was no evidence of any clinically significant haematotoxicity, neither per patient nor by course. Details per course are given in Table 2.

Table 2
Haematological toxicity maximum NCI-CTC grades per course regardless relationship to study drug

		Grades				
Parameter	Dose (mg)	0	1	2	3	4
Haemoglobin	25	0	6	0	0	0
	50	0	2	0	0	0
	100	0	3	2	0	0
	150	1	2	0	0	0
	200	0	5	2	0	0
Platelets	25	6	0	0	0	0
	50	2	0	0	0	0
	100	5	0	0	0	0
	150	3	0	0	0	0
	200	7	0	0	0	0
White blood cell count	25	6	0	0	0	0
	50	2	0	0	0	0
	100	5	0	0	0	0
	150	1	0	0	0	0
	200	7	0	0	0	0
Neutrophils	25	6	0	0	0	0
	50	2	0	0	0	0
	100	4	1	0	0	0
	150	3	0	0	0	0
	200	7	0	0	0	0
Lymphocytes	25	6	0	0	0	0
	50	2	0	0	0	0
	100	4	0	1	0	0
	150	3	0	0	0	0
	200	2	0	4	1	0

NCI-CTC, National Cancer Institute-Common Toxicity Criteria.

3.2.2. Non-haematological toxicities

Almost all of the patients developed some degree of reversible hepatotoxicity (Table 3). Hepatotoxicity was dose-dependent (Table 4). Only the first patient, when treated with the initial dose, of 25 mg total dose, did not experience an increase in the liver enzymes. Nevertheless, when this patient inadvertently did receive a dose of 100 mg of the drug, CTC grade 2 was reached for the GPT and GOT levels. Beginning from the second dose level of 50 mg total dose liver enzymes increased to pathological values, a finding in all but 2 further patients. In patient #4, who was treated with 200 mg total dose, who started with already increased liver enzymes due to tumour involvement of the liver, it is to be speculated whether this was due to toxicity or because of the progression of the disease in the liver after the first cycle of therapy.

Besides hepatotoxicity, the following other non-haematological toxicities were registered: Nausea and vomiting was observed in 3 patients, but only in a single patient (at the 200 mg single dose level) the grade 2 toxicity observed at the first course deteriorated to a grade 3 nausea at the second cycle. In that same and only patient, fatigue CTC grade 2 was classified as probably related by the treating investigator.

The DLT was reached at a dose of 200 mg BIBX 1382 BS per day. It consisted of hepatotoxicity, as evidenced by grade 3 GOT and GPT elevations which, although reversible, is not a side-effect which can be tolerated for an oral drug to be given on a daily basis. From the animal

data, it is hypothesised that this hepatotoxicity is caused by a metabolite of BIBX 1382 BS. On the basis of this hepatic toxicity, it was decided to close down the study. A MTD of BIBX 1382 BS was therefore not fully investigated; the study was stopped before the MTD was found. Consequently, most of the above referred study objectives (to determine MTD, to propose a safe dose and to select an optimal dose for phase II studies) were not achieved.

3.3. Antitumour activity

No major or minor response(s) were observed. Five patients (45%) experienced progression of their disease (PD), 2 patients (18%) even before the first time-point of disease evaluation, i.e. early progressive disease (EPD); an additional 4 patients (36%) were not evaluable for response, in 3 out of the 4 cases because the patients received less than a full cycle of therapy (patients #4, #10, #11); patient #6 showed clear progression of her pancreatic cancer under BIBX 1382 BS, while continuing antihormonal anticancer treatment against a previously detected and treated breast cancer.

3.4. Pharmacokinetic studies

Detailed data are not shown. For the i.v. doses, the dose normalised AUC_{0-24h} (or mean value for this parameter when more patients at the same dose level) ranged from approximately 3.3 to 13.4 ng h/ml for the studied dose range. The individual $t_{1/2}$ ranged from 6.4 to 13.9 h, without any dose dependency. The dose

Table 3
Hepatotoxicity associated with the administration of BIBX 1382 BS (the worst grade per dose level, per patient is given)

Patient	Number of courses	Dose level (mg)	Hepatotoxicity (NCI–CTC grade)					Serious adverse events (SAEs) besides hepatotoxicity (relation of SAEs to study drug)	Best response	Tumour entity
			GOT	GPT	AP	GGT	Bili			
#1	6	25	0	0	0	0	0	No SAEs (hepatotoxicity initially considered unrelated)	PD	Renal cancer
	1	100	2	2	1	2	0			
#2	2	50	1	2	2	3	2	Nausea, emesis, epigastric pain G2 (unlikely)	EPD	Renal cancer
#3	2	100	1	1	1	3	0	Sinusitis, laryngitis G3; urinary tract infection moderate (not related); pain severe (not related)	PD	Breast cancer
#4	1	200	NA	NA	NA	NA	NA	No SAEs; pre-existing elevated liver function tests due to tumour involvement of the liver	NE	Breast cancer
#5	1	200	3	3	1	2	0	No SAEs	PD	Gastric cancer
	1	100	2	2	0	1	0			
#6	2	200	2	2	2	3	0	Ileus (not related)	NE	Pancreatic cancer
#7	1	200	3	3	1	3	0	None	PD	Melanoma
	1	100	1	1	1	3	0			
#8	2	200	2	3	2	4	3	Increase of pre-existing elevated AP and GGT; patient refusal after disclosure of study termination	PD	Colon cancer
#9	1	150	3	3	0	2	0	None	EPD	Melanoma
	1	100	1	2	0	2	0			
	1	50	0	0	0	1	0			
#10	1	150	1	2	0	2	0	Patient refusal after disclosure of study termination; increase of pre-existing elevated liver function tests due to tumour involvement of the liver	NE	Renal cancer
#11	1	150	0	0	1	1	0	No SAEs; only i.v. dose applied; no change of pre-existing elevated AP and GGT; patient refusal after disclosure of study termination	NE	Lung cancer

GOT = ASAT, aspartate aminotransferase; GPT = ALAT, alanine aminotransferase; AP, alkaline phosphatase; GGT, gamma-glutamyl transferase; PD, progressive disease; EPD, early progressive disease; NE, not evaluable; NA, not applicable; NCI-CTC, National Cancer Institute–Common Toxicity Criteria; Bili, bilirubin; i.v., intravenous.

normalised (mean) plasma concentration at the end of the infusion ranged from 0.6 to 6.5 ng/ml, also without any dose dependency, except for the lowest dose, which had the lowest C_{end}. The clearance (CL) (mean) ranged from 25 to 55 ml/min/kg, with the highest value for the lowest dose. Renal excretion (mean) was very low; maximum 1.2% of the dose was excreted via this route within 48 hours after drug administration.

After oral dosing, the dose normalised AUC_{0-24h} ranged from 0.036 to 0.97 ng h/ml without a clear dose dependency, except for the lowest dose which had the lowest AUC_{0-24h}. No dose dependency was found for the $t_{1/2}$ values, which varied from 2.0 to 10.3 h. The dose normalised maximum plasma concentration was very low, ranging from 0.009 to 0.16 ng/ml. The absolute bioavailability of the compound was also very low, between 1.8 and 12.3% (mean value over all patients: 4.8%), again without any obvious dose dependency.

Table 4
Biochemistry maximum NCI-CTC grades per course regardless relationship to study drug

		Grades				
Item	Dose (mg)	0	1	2	3	4
Bilirubin	25	6	0	0	0	0
	50	1	1	0	0	0
	100	5	0	0	0	0
	150	3	0	0	0	0
	200	4	1	1	1	0
Alkaline phosphatase	25	6	0	0	0	0
	50	0	0	2	0	0
	100	1	4	0	0	0
	150	2	1	0	0	0
	200	0	3	3	1	0
ASAT (GOT)	25	6	0	0	0	0
	50	1	1	0	0	0
	100	1	2	2	0	0
	150	1	1	0	1	0
	200	0	1	3	3	0
ALAT (GPT)	25	2	0	0	0	0
	100	0	3	2	0	0
	150	1	0	1	1	0
	200	0	2	1	4	0
GGT	25	6	0	0	0	0
	50	0	0	1	1	0
	100	0	1	2	2	0
	150	0	1	2	0	0
	200	0	0	2	3	2
Creatinine	25	3	3	0	0	0
	50	0	2	0	0	0
	100	1	4	0	0	0
	150	1	2	0	0	0
	200	2	3	2	0	0

ASAT, aspartate aminotransferase; GOT, glutamic-oxaloacetic transaminase; ALAT, alanine aminotransferase; GPT, glutamic-pyruvic transaminase; GGT, gamma-glutamyl transferase; NCI-CTC, National Cancer Institute-Common Toxicity Criteria.

The urinary excretion was low, maximum 0.05% of the dose in 48 h after administration.

The pharmacokinetic parameters of BIBX 1382 BS indicate that i.v. administration of the drug does not lead to very high plasma concentrations and exposure of the drug. After oral administration the intra-individual variation in pharmacokinetics was quite high. Overall, no dose dependency in the pharmacokinetics was observed, with the exception of the lowest dose, which was for some parameters deviating from the data obtained with the other doses.

The absolute bioavailability of the compound after oral administration was variable and low, with an overall mean value of approximately 5%.

To investigate the effect of metabolic enzymes on the low oral bioavailability, grapefruit juice was administered before the oral dose in one patient. However, this extra treatment did not result in any change in the pharmacokinetic parameters compared with the data obtained in the same patient without the administration of grapefruit juice. $AUC_{0\text{-last}}$ with and without grapefruit juice were 141 and 127 ng.h/ml, respectively.

In addition, no effect could be observed when comparing the administration of an oral solution of the drug and a tablet formulation, indicating that poor dissolution of the tablet was not responsible for the low bioavailability. A caffeine-containing soft drink was administered immediately after the ingestion of the liquid formulation developed for i.v. injection for correction of taste of the latter as well as for keeping the pH-value of the upper intestine below 5.

The plasma concentrations of the metabolite BIBX 1330 BS after i.v. as well as oral dosing of the parent compound were very low and were not very reproducible, indicating that there was no major contribution from this compound to the overall effect of the drug.

Since the concentration of BIBX 1382 BS was very low and hardly detectable in all of the patients, the effect of AGP has not been further examined.

All patients, except for patient #9, were found to be extensive metabolisers with regard to CYP2D6.

Since concentrations and bioavailability of BIBX 1382 BS were very low, it is not expected that the patient's *CYP2D6* genotype plays any significant role in the toxicity or efficacy of this compound.

4. Discussion

The novel pyrimido-pyrimidine BIBX 1382 BS was selected on the basis of its preclinical profile for further development. In most EGFR-dependent tumour models, an oral dose of 30 mg/kg/day of BIBX 1382 BS proved sufficient for tumour growth control. This translates into 90 mg/m^2/day or 150 mg/day in humans. Nevertheless, higher doses than 90 mg/m^2/day may be

needed in humans since tumour patients have at least 5- to 10-fold higher alpha-1 acid glycoprotein (AGP) plasma levels than mice. AGP is expected to lower the potency of BIBX 1382 BS since BIBX 1382 BS and its metabolite BIBX 1330 are bound in the plasma to AGP. In EGFR-dependent tumour models, the effective steady-state plasma concentrations of the parent drug BIBX 1382 BS at the above indicated dosage (of 30 mg/kg/day) were between approximately 20 ng/ml (C_{min}) and 1200 ng/ml (C_{max}). The steady-state plasma concentrations of the metabolite BIBX 1330 were between approximately 40 ng/ml (C_{min}) and 300 ng/ml (C_{min}) notwithstanding the different binding to AGP in the plasma of mice and individual tumour patients. In addition, it is known that the drug is metabolised by the isoenzyme CYP2D6, resulting in poor and extensive metabolisers. Poor metabolism of BIBX 1382 BS may result in accumulation and/or undesired drug effects.

Taking into account all the above arguments and problems, respectively, the decision was taken to develop the compound using real time pharmacokinetic analysis during the stage I part of the study.

A total of 11 patients were entered in the study. Dose levels of 25, 50, 100, 200, and 150 mg (in this order) were investigated. The doses for the subsequent patients were determined on the basis of pharmacokinetic and adverse event data from the previous patients.

The pharmacokinetic data after single i.v. and single oral dosing showed low bioavailability (5%) of BIBX 1382 BS after oral dosing compared with i.v. administration. Bioavailability was not greatly altered by the administration of an oral liquid formulation (deleting the dissolution step in the process of absorption from the gastro-intestinal tract) in patient #3 (dose of 100 mg) by using the liquid formulation for oral ingestion. Correction of taste while keeping the pH value of the upper intestine below 5— in order to simulate the condition for maximal absorption with decreasing solubility of the drug at pH >6 in rodents— was attempted via administration of a caffeine-containing soft drink to be swallowed immediately thereafter. Nevertheless, this procedure did not result in the intended effect of increased plasma concentration as demonstrated by the real time pharmacokinetic data available to us before defining the next step of further drug development. Thus, limited dissolution of the tablets is not expected to be responsible for the low bioavailability.

Bioavailability was not greatly altered by the co-administration of grapefruit juice, known to inhibit CYP 3A4 [11], the main intestinal drug-metabolising enzyme, to protect the medication from being destroyed before it is absorbed. Only a slightly, but not adequately increased AUC was detected after the grapefruit ingestion in patient #4 who was exposed to the dose level of 200 mg total dose. This patient also had extensive liver metastases. We concluded that gastro-intestinal degradation by CYP 3A4 is not responsible for the low bioavailability.

The most significant adverse event, in most cases considered to be at least possibly related to study drug, was liver toxicity, evidenced by elevations in liver transaminases. Grade 3 elevations of GOT and GPT occurred at the dose level of 200 mg and of 150 mg, with elevations up to grade 2 occurring in patients administered 100 mg BIBX 1382 BS daily.

Whether a higher degree of involvement of liver metastases in patient #4 and of 2 further patients (#6, #8), who also received the 200 mg/day dosage or the corresponding liver toxicity of the drug at that dose level were the reason for the higher C_{max} and AUC values, found in these patients is a matter of speculation. Nevertheless, even the highest C_{max} and AUC values reached were below or at the lower threshold levels known to be necessary for antitumoural activity from preclinical test systems.

Meanwhile, accompanying preclinical experiments disclosed that BIBX 1382 BS is metabolised by a hepatic aldehyde oxidase to M404/9.3, exceeding the plasma concentration of the parent drug BIBX 1382 BS by an order of magnitude. This metabolic-mechanism was only very recently observed in rhesus monkeys, but not in other animal species and was therefore not known from the preclinical experiments in mice and rats and does not even exist in dogs. The activity of this metabolite is decreased by 500× (in a cellular assay) and by 1000× (in a kinase assay) compared with the parent compound BIBX 1382 BS. Theoretically, the metabolic inactivation might have been saturable. Nevertheless, because of the liver toxicity of BIBX 1382 BS and/or its metabolite(s) it was not possible to increase the dose of BIBX 1382 BS to dose levels necessary to reach pharmacologically relevant plasma concentrations in man. From animal experiments, it might be extrapolated that average concentrations of 100 ng/ml would be needed to reduce tumour growth. In this study, C_{max} values varied between one hundredth (2–3 ng/ml) and one tenth (10–20 ng/ml) of that concentration at oral doses of 100 and 200 mg, respectively; the trough levels at a dose of 200 mg were around 2–3 ng/ml in 1 patient and between 0.2 and 0.4 ng/ml in another.

Based on these data, mainly generated via real-time pharmacokinetic analyses, and the putative interpretation of accompanying preclinical research, the benefit/risk ratio was considered to be to unfavourable to justify further development of this drug.

This study was not able to add another attractive compound to the pharmacological armamentarium of anticancer treatment. However, only the use of a real time pharmacokinetic analysis allowed the termination of the development of this compound within less than a year to be decided, resulting in only 11 patients being treated with an ineffective drug.

Acknowledgements

BIBX 1382 BS was supplied by Boehringer Ingelheim, Biberach, Germany. The authors thank the scientists of Boehringer Ingelheim Austria and Boehringer Ingelheim Pharma KG, Germany, for providing pharmacological and toxicological data.

References

1. Van der Geer P, Hunter T, Lindberg RA. Receptor protein-tyrosine kinases and their signal transduction pathways. *Ann Rev Cell Biol* 1994, **10**, 251–337.
2. Davies DE, Chamberlin SG. Targeting the epidermal growth factor receptor for therapy of carcinomas. *Biochem Pharmacol* 1996, **51**, 1101–1110.
3. Modjtahedi H, Eccles S, Sandle J, Box G, Titley J, Dean C. Differentiation of immune destruction: two pathways for therapy of squamous cell carcinomas with antibodies to the epidermal growth factor receptor. *Cancer Res* 1994, **54**, 1695–1701.
4. Fan Z, Massui H, Altas I, Mendelsohn J. Blockade of epidermal growth factor receptor function by bivalent and monovalent fragments of 225 anti-epidermal growth factor receptor monoclonal antibodies. *Cancer Res* 1993, **53**, 4322–4328.
5. Levitzki A. Signal transduction therapy. *Eur J Biochem* 1994, **226**, 1–13.
6. Egeblad M, Mortensen OH, van Kempen LC, Jaattela M. BIBX1382BS, but not AG1478 or PD153035, inhibits the Erb kinases at different concentrations in intact cells. *Biochem Biophys Res Commun* 2001, **281**, 25–31.
7. Kremer JMH, Wilting J, Janssen LHM. Drug binding to human alpha-1-acid glycoprotein in health and disease. *Pharm Rev* 1988, **40**, 1–47.
8. WHO. *Handbook for Reporting Results of Cancer Treatment, vol 48*. Geneva, WHO, 1979.
9. Heinzel G, Woloszczak R, Thomann P. *TopFit, Version 2.0; Pharmacokinetic and Pharmacodynamic Data Analysis System for the PC*. Stuttgart, G. Fischer Verlag, 1993.
10. Beal SL, Sheiner LB, Boeckmann AJ. *NonMem Version V*. San Francisco, University of California, 1998.
11. Soldner A, Christians U, Susanto M, Wacher VJ, Silverman JA, Benet LZ. Grapefruit juice activates P-glycoprotein-mediated drug transport. *Pharm Res* 1999, **16**, 478–485.