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PHASE I STUDIES

Pharmacokinetics and excretion of ^{14}C-lenvatinib in patients with advanced solid tumors or lymphomas

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Summary Lenvatinib is an orally available multi-targeted tyrosine kinase inhibitor with anti-angiogenic and antitumor activity. To get more insight into the disposition of lenvatinib, a mass balance study was performed in patients with advanced solid tumors. A single oral 24 mg (100 μCi) dose of ^{14}C-lenvatinib was administered to six patients, followed by collection of blood, plasma, urine and feces for 7 to 10 days. The collected material was analyzed for total radioactivity, unchanged lenvatinib and selected metabolites. The safety and antitumor effect of a daily oral dose of 24 mg non-labeled lenvatinib were assessed in the extension phase of the study. Peak plasma concentrations of lenvatinib and total radioactivity were reached 1.6 and 1.4 h after administration, respectively, and their terminal phase half-lifes were 34.5 and 17.8 h, respectively. Unchanged lenvatinib systemic exposure accounted for 60 % of the total radioactivity in plasma. Peak concentrations of the analyzed metabolite were over 700-fold lower than the peak plasma concentration of lenvatinib. Ten days after the initial dose, the geometric mean (± CV) recovery of administered dose was 89 % ±10 %, with 64 % ±11 % recovered in feces and 25 % ±18 % in urine. Unchanged lenvatinib in urine and feces accounted for 2.5 % ±68 % of the administered dose, indicating a major role of metabolism in the elimination of lenvatinib. In conclusion, lenvatinib is rapidly absorbed and extensively metabolized, with subsequent excretion in urine and, more predominantly, in feces. Additionally, lenvatinib showed acceptable safety and preliminary antitumor activity.

Keywords Lenvatinib · E7080 · Mass balance · Excretion · Pharmacokinetics

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Introduction

Angiogenesis, the formation and proliferation of blood vessels, is essential for tumor progression and metastasis and is, therefore, an important target to arrest tumor growth. The onset of angiogenesis, the so-called "angiogenic switch", is a discrete step in tumor propagation and refers to an imbalance in pro-angiogenic and anti-angiogenic factors, in favor of the first [1]. Examples of pro-angiogenic factors are vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). Binding of these growth factors with their specific receptor tyrosine kinases on the surface of endothelial cells leads to activation of the kinases, causing a

cascade of cellular signals and resulting in cellular responses such as proliferation and migration [2]. Over the last decade, several anticancer drugs, targeting various spectra of receptor tyrosine kinases, have been developed and approved. Multi-targeted tyrosine kinase inhibitors have shown notable clinical results [3].

Lenvatinib (E7080) is an investigational orally active inhibitor of multiple receptor tyrosine kinases, including VEGF, FGF, PDGF and stem cell factor (SCF) receptors [4]. Apart from inhibiting angiogenesis by targeting endothelial cells [4–7], lenvatinib exerts a direct effect on tumor cells, by inhibiting their migration and invasion [8]. Promising antitumor effects of lenvatinib were observed in Phase I trials [9, 10], leading to a number of disease-specific phase 2 and phase 3 trials with lenvatinib as a single agent or in combination with other anticancer agents.

To get more insight into the absorption, distribution, metabolism and excretion of lenvatinib in humans, a mass balance study was performed employing ^{14}C-radiolabelled lenvatinib. The primary objectives of this study were to determine the pharmacokinetics of lenvatinib and its excretion balance in patients with advanced tumors or lymphomas. To achieve this, a single dose of ^{14}C-lenvatinib was administered to patients, followed by collection of blood samples and excreta. The samples were analyzed for total radioactivity, unchanged lenvatinib and four lenvatinib metabolites (Fig. 1), for which validated quantitative assays were available [11]. The secondary objectives of this study were to assess the safety of lenvatinib when given continuously as a single daily dose of 24 mg and to explore the antitumor activity of lenvatinib.

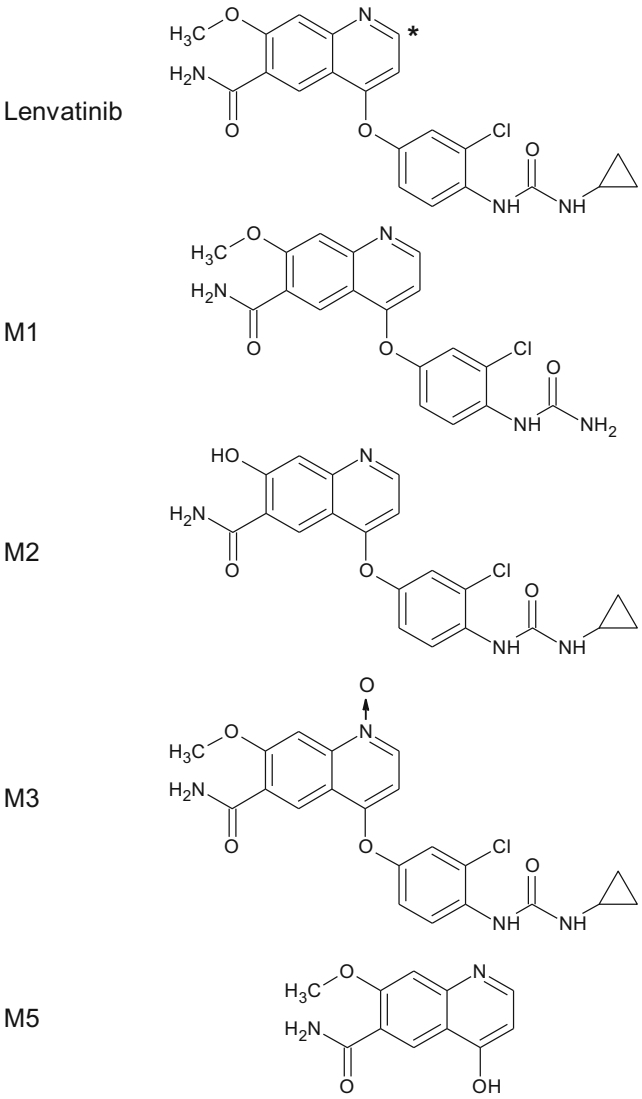


Fig. 1 Chemical structure of ^{14}C-lenvatinib, with the asterisk indicating the position of the ^{14}C-label, and lenvatinib metabolites: products of decyclopropylation (*M1*), demethylation (*M2*), N-oxidation (*M3*) and *O*-dearylation (*M5*)

Materials and methods

Study design

This was a Phase I, open-label, single centre (The Netherlands Cancer Institute, Amsterdam, the Netherlands) study, which enrolled six patients with advanced solid tumors or lymphomas. The study was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki. The protocol was approved by the Netherlands Cancer Institute Independent Ethics Committee.

The study comprised two phases: the study phase, designed to fulfill the primary objectives (determination of pharmacokinetics and excretion), and the extension phase, aimed to fulfill the secondary objectives (assessment of safety and efficacy). On day 1 of the study phase, each patient received a single administration of approximately 24 mg ^{14}C-labeled lenvatinib (approximately 100 μCi, 3.7 MBq) as an oral dosing solution. Blood, urine and feces were collected during the subsequent 7 days, and, if necessary, collection of excreta continued on an out-patient basis until the radioactivity in urine and feces samples was <1 % of the administered radioactivity. In the extension phase, starting after collection of the last study sample, patients received a 24 mg oral dose of non-radiolabeled lenvatinib once daily in continuous 28-day treatment cycles.

Patients

Patients aged 34 to 64 years with a histologically or cytologically confirmed advanced solid tumor or lymphoma, who were unsuitable or failed existing therapies, were enrolled in this study. Patients had an Eastern Cooperative Oncology Group (ECOG) performance status ≤2. Any previous

treatment (including surgery and radiotherapy) was completed at least 4 weeks prior to study entry with any acute toxicity resolved. Patients were excluded if they had the following laboratory parameters: hemoglobin <9 g/dL, neutrophils $<1.5\times10^9$/L, platelets $<100\times10^9$/L, prothrombin time (PT) (or international normalized ratio [INR]) and partial prothrombin time (PTT) >1.5 × the upper limit of normal (ULN), serum bilirubin >1.5 × ULN, other liver parameters >3 × ULN and creatinine clearance <60 mL/min. Other exclusion criteria included brain or subdural metastases (unless the therapy was completed and signs and/or symptoms were stable for at least 4 weeks prior to study start), meningeal carcinomatosis, marked baseline prolongation of QT/QTc interval, pregnancy and breast-feeding, proteinuria >1+ on bedside testing, history of gastrointestinal malabsorption, bleeding or thrombotic disorders or use of an anticoagulant, such as warfarin, with a therapeutic international normalized ratio, poorly controlled hypertension or hypertension at screening, previous lenvatinib therapy, and any significant disease, disorder or condition that, in the investigator's opinion, excluded the patient from the study.

Study medication

For the study phase, individual ^{14}C-lenvatinib dosing solutions were prepared by mixing non-labeled lenvatinib mesylate powder (chemical purity 98.2 % Eisai Co. Ltd., Ibaraki, Japan) dissolved in 3 mM hydrochloric acid solution with a ^{14}C-lenvatinib solution (chemical and radiochemical purity ≥98.2 %, GE Healthcare Life Sciences, Cardiff, United Kingdom) to a final concentration of 2 mg/mL lenvatinib as anhydrous free base with a radioactivity of around 8.3 μCi/mL. A small portion of the mixture was used to analyze the exact radioactive concentration. A volume of 12 mL containing 24 mg ^{14}C-lenvatinib (100 μCi) was orally administered to the patient from a syringe. The syringe was washed with water, which was also administered orally. The residual radioactivity in the syringe was measured after flushing it and used to calculate the actual administered dose.

In the extension phase, the daily dose of 24 mg lenvatinib was taken as 2 tablets of 10 mg and 1 tablet of 4 mg.

Total radioactivity analysis

Total radioactivity in (i) plasma (of venous blood collected pre-dose, and 15 and 30 min and 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h after ^{14}C-lenvatinib administration), (ii) whole blood (collected pre-dose, and 15 and 30 min and 1, 2, 4, 8, 24, 72 and 168 h after ^{14}C-lenvatinib administration), (iii) urine (collected pre-dose, over 6-h periods during the first 48 h after ^{14}C-lenvatinib administration and over 24-h periods thereafter) and (iv) fecal samples (collected per portion, pre-dose and post-dose until radioactivity was <1 % of administered dose) was determined by liquid scintillation counting.

To this end, aliquots of whole blood (0.2 mL) and fecal homogenates (0.2 mL of homogenate consisting of feces-water 1:3 *w/v*) were prepared as described elsewhere [12], using Solvable (Perkin Elmer, Waltham, MA, USA), 30 % hydrogen peroxide plus either 0.1 M EDTA (blood) or isopropanol (feces). The resulting decolorized and dissolved blood and fecal samples were, in addition to non-processed plasma (0.2 mL) and urine (1 mL) aliquots, mixed with 10 mL liquid scintillation cocktail (Ultima Gold, Perkin Elmer) and counted on a Tri-Carb 2800TR liquid scintillation counter (Perkin Elmer). The lower limit of quantification (LLOQ) was approximated using 5 % as a maximum of uncertainty (%2s) in the gross count value, with 95 % confidence limits. The formula used is a simplified form of the formula of %2s described by Zhu et al. [13]:

$$\%2s = \frac{200}{\sqrt{CPM_A T}} \tag{1}$$

Wherein CPM_A is the activity of the sample in counts per minute and T the counting time in min. Samples were counted until a %2s below 1 % was achieved, with a maximum counting time of 60 min. Using Eq. (1), with a maximum %2s of 5 % and a maximum counting time of 60 min, the LLOQ was calculated to be 27 CPM (after background subtraction). For this low number the number of counts per minute is almost equal to the number of disintegrations per minute (DPM). The value of 27 DPM was finally converted into an LLOQ in the various matrices, using formula (2):

$$LLOQ = \frac{\frac{26.7}{60\times 37000}\times\frac{D_{Adm}\times 10^6}{A_{Adm}}}{m_s}\left(\frac{m_{homogenate}}{m_{faeces}}\right)^{*} \tag{2}$$

*This factor only applies for fecal samples, to correct for the dilution facor

Wherein D_{Adm} and A_{Adm} are the total (=labeled plus non-labeled) administered dose (as a free base) and the administered activity in μCi, respectively, wherein m_s is the average mass of samples from a specific matrix (0.2 g for plasma, whole blood and fecal homogenates and 1 g for urine) and wherein m_{feces} and $m_{homogenate}$ are the masses of the individual fecal portions and homogenate portions, respectively.

The exact LLOQ was therefore dependent on the radioactive concentration of the individual dosing solutions and varied slightly between patients. In plasma and blood, the LLOQ was ~13 ng eq/mL, in urine ~2.5 ng eq/mL and in feces ~50 ng eq/mL.

Quantitative bioanalysis

All samples that were analyzed for total radioactivity were additionally analyzed using validated liquid chromatography tandem mass spectrometry (LC-MS/MS) assays, described elsewhere [11]. Unchanged lenvatinib was quantified in all matrices and the lenvatinib metabolites M1, M2, M3 and M5 (of which reference standards were provided by Eisai Co. Ltd, Tsukuba, Japan) (Fig. 1) were quantified in plasma, urine and feces. The naming of the metabolites was based on preclinical drug metabolism studies.

Briefly, plasma, urine and feces samples were extracted with acetonitrile and separated on a 50×2.1 mm I.D. XTerra MS C18 column with gradient elution. Whole blood samples were extracted with diethyl ether and separated on a 150× 2.1 mm I.D. Symmetry Shield RP8 column. Detection was performed in multiple reaction monitoring mode on a API3000 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA). The LLOQ of lenvatinib and its metabolites in plasma and of lenvatinib in whole blood was 0.25 ng/mL. In urine, the LLOQ of lenvatinib, M1, M2 and M3 was 1.0 ng/mL and the LLOQ of M5 was 2.5 ng/mL. In feces, the LLOQ of lenvatinib was 0.1 μg/mL and the LLOQ of M1, M2, M3 and M5 was 0.02 μg/mL.

Quality control samples were prepared and analysed together with the study samples and acceptance criteria for bioanalytical data during routine drug analysis, as the FDA recommends [14].

Pharmacokinetic and statistical analysis

Individual plasma/blood concentration-time data were analyzed using non-compartmental analysis with WinNonlin™ Professional (version 5.1.1 and 5.2, Pharsight Corp, Mountain View, CA, USA). The pharmacokinetic parameters calculated for lenvatinib in plasma or blood included the following: maximum concentration (C_{max}), terminal phase half-life ($t_{½}$), area under the plasma/blood concentration time curve (AUC), renal clearance (CL_r), apparent oral clearance (CL/F) and apparent volume of distribution (V_z/F). Also the cumulative percentage of the ^{14}C-lenvatinib dose recovered in urine and/or feces as total radioactivity, lenvatinib, M1, M2, M3 or M5 (Fig. 1) was determined. Descriptive statistics, including n, geometric mean (which reduces the impact of potential outliers) and coefficient of variation (CV) were used to summarize the drug and metabolite concentration values and pharmacokinetic parameters.

Safety and efficacy assessments

The safety/tolerability of lenvatinib was assessed throughout the study by evaluation of physical examinations, ECOG performance status, vital signs, clinical laboratory tests, electrocardiograms, concomitant medications, adverse events and serious adverse events. Adverse events were graded using CTCAE v3.0.

The efficacy of lenvatinib was evaluated by assessment of tumor response in accordance with Response Evaluation Criteria in Solid Tumors (RECIST, version 1.0). In patients evaluable according to RECIST, a best response was assigned by the investigator. Responses (complete response [CR] or partial response [PR]) and stable disease (SD) were confirmed according to RECIST.

Results

Patients

Six patients were enrolled (three male and three female), with a median age of 49 years (range 34–64), a mean weight of 95.7 kg (range 60–166), a mean height of 179.8 cm (range 168–210) and a mean body surface area of 2.17 m^2 (range 1.7–3.1).

Pharmacokinetics

Plasma and whole blood concentration curves of total radioactivity and lenvatinib over 168 h after administration of the ^{14}C-lenvatinib dose are presented in Fig. 2. Also the plasma concentrations of M2 versus time are shown in Fig. 2; the plasma concentrations of the other metabolites were below the lower limit of quantification (0.25 ng/mL). The mean lenvatinib C_{max} is approximately 88 % of the C_{max} for total radioactivity (Fig. 2, Table 1).

Table 1 provides a summary of the pharmacokinetic parameters. The peak concentration of total radioactivity and lenvatinib in plasma and whole blood was reached between 1.4 and 1.8 h after oral administration of the ^{14}C-lenvatinib solution. Based on $AUC_{0\text{-}24h}$, the blood concentrations of total radioactivity and lenvatinib were approximately 29 and 36 %, respectively, lower than the plasma concentrations. Unchanged lenvatinib accounted for 60 % of the total radioactivity in plasma and 64 % of the total radioactivity in blood.

Plasma concentrations of the metabolites M1, M2, M3 and M5 were generally below the lower limit of quantification of the assay. Only peak concentrations of M2 (n=6) and M5 (n= 1) were quantifiable. All peak metabolite concentrations were over 700-fold lower than the peak plasma concentration of lenvatinib.

Based on plasma concentrations of unchanged lenvatinib, the terminal phase half-life of lenvatinib was 34.5 h, the apparent oral clearance was 6.7 L/h, the renal clearance was 0.042 L/h and the apparent volume of distribution was 336 L.

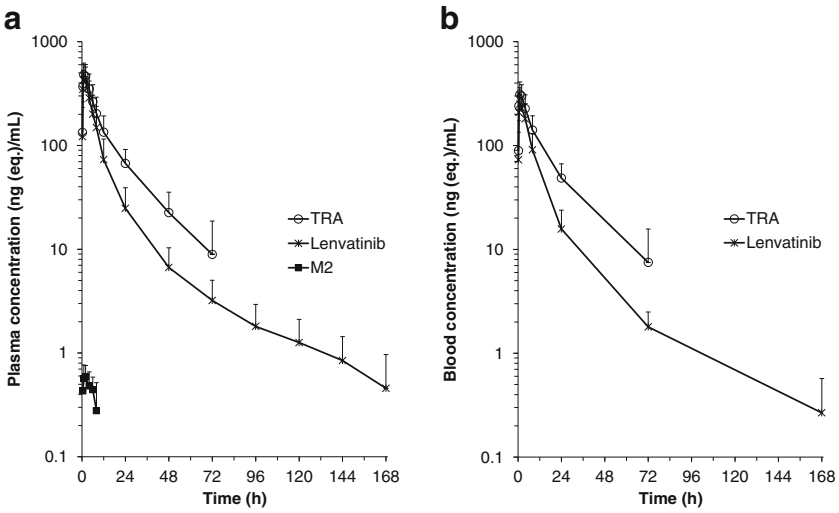


Fig. 2 Mean (+ standard deviation) concentration-time curves of total radioactivity (*TRA*), lenvatinib and M2 in plasma (**a**) and of TRA and lenvatinib in whole blood (**b**) following a single oral administration of 24 mg (100 μCi) ^{14}C-E7080. The mean values at time points with one or more individual values below LLOQ were calculated by assigning 0 for each value below LLOQ

Excretion balance

For all 6 patients, urine and feces was collected as planned during the first 7 days after administration of ^{14}C-lenvatinib. The last urine and fecal samples that were collected in this period generally contained <1 % of the administered radioactivity. For two patients the collection of feces continued to Day 9 and Day 11.

Figure 3a shows the mean cumulative urinary, fecal and total recovery of total radioactivity during the first 240 h after ^{14}C-lenvatinib administration. Figure 3b, c and d show the mean cumulative percentage recovery of dose as unchanged lenvatinib, M1, M2, M3, M5 and total radioactivity in urine, in feces and in urine and feces combined, respectively. The total recovery after 240 h was approximately 89 % of the administered dose (Table 2), with approximately 64 % in feces and 25 % in urine.

Table 1 Plasma and whole blood pharmacokinetic parameters for total radioactivity, lenvatinib and its metabolites M1, M2, M3 and M5 following a single oral dose of 24 mg (100 μCi) of ^{14}C-lenvatinib

Parameter	TRA blood (*n*=6)	TRA plasma (*n*=6)	Lenvatinib blood (*n*=6)	Lenvatinib plasma (*n*=6)	M1 plasma (*n*=0)	M2 plasma (*n*=6)	M3 plasma (*n*=0)	M5 plasma (*n*=1)
C_{max} (ng/mL)	313.4 (39.1)	485.2 (37.1)	248.1 (45.9)	426.8 (46.6)	<LLOQ	0.6106 (23.9)	<LLOQ	0.300
t_{max} (h)	1.42 (0.95–2.12)	1.42 (0.95–2.12)	1.80 (0.95–4.02)	1.60 (0.95–2.12)	NC	1.42 (0.95–4.02)	NC	0.25
$AUC_{0\text{-}24h}$ (μg.h/mL)[a]	2.97 (39.9)	4.20 (42.0)	1.89 (45.1)	2.93 (48.1)	NC	NC	NC	NC
$AUC_{0\text{-}t}$ (μg.h/mL)[a]	3.54 (53.4)	5.22 (55.0)	2.21 (47.1)	3.44 (49.7)	NC	NC	NC	NC
$AUC_{0\text{-}\infty}$ (μg.h/mL)[a]	3.73[b] (60.4)	5.78 (48.2)	1.52[c] (59.0)	3.47 (50.0)	NC	NC	NC	NC
$t_{½}$ (h)	10.8[b] (32.6)	17.8 (39.4)	11.8[c] (3.95)	34.5 (25.1)	NC	NC	NC	NC
CL/F (L/h)	ND	ND	15.0[c] (65.8)	6.74 (49.5)	NC	NC	NC	NC
CL_r (L/h)	ND	ND	0.065 (114)	0.042 (140)	NC	NC	NC	NC
V_z/F (L)	ND	ND	255[c] (70.9)	336 (36.7)	NC	NC	NC	NC

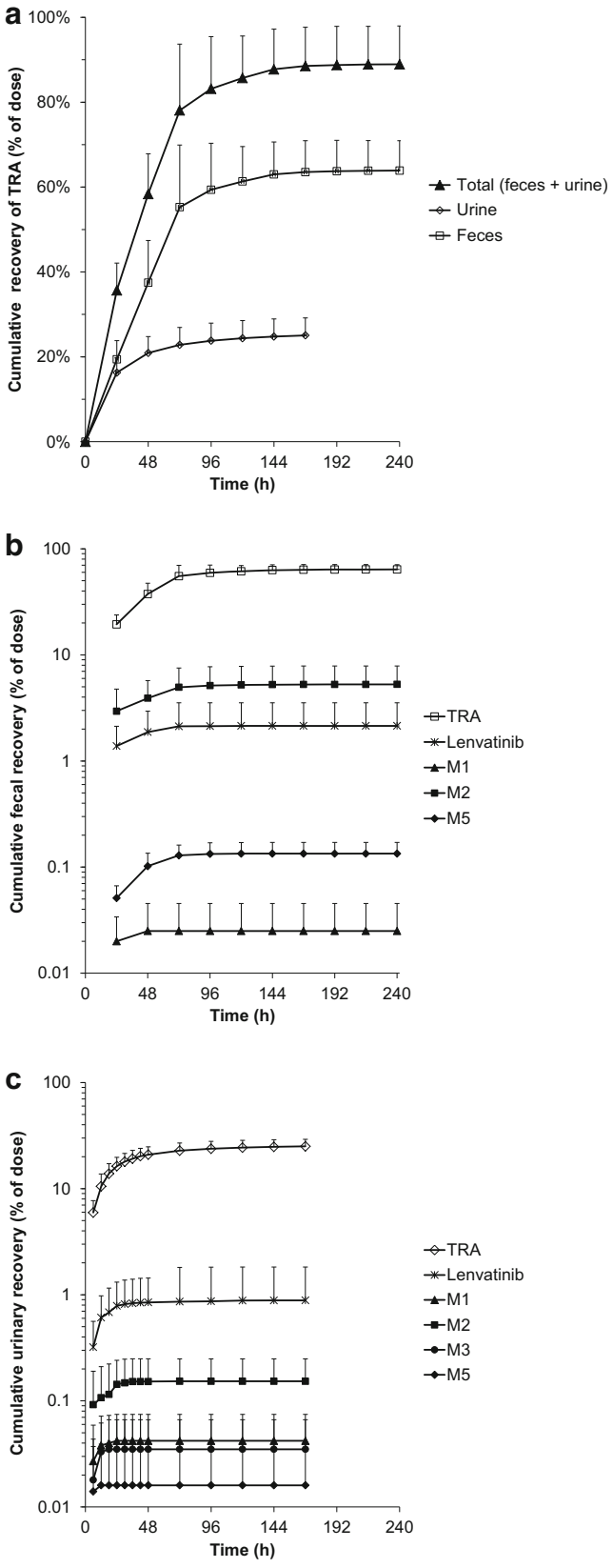
Values are presented as geometric means (CV%), except for t_{max}, which is presented as geometric mean (range)

TRA total radioactivity, *n* number of patients with quantifiable data, C_{max} maximum blood/plasma concentration, t_{max} time at which maximum blood/plasma concentration was reached, $AUC_{0\text{-}24h}$ area under the blood/plasma concentration-time curve from time zero to 24 h, $AUC_{0\text{-}t}$ area under the blood/plasma concentration-time profile from time zero to last observed quantifiable concentration, $AUC_{0\text{-}\infty}$ area under the blood/plasma concentration-time curve from time zero up to infinity, $t_{½}$ terminal exponential half-life, *CL/F* oral clearance, CL_r renal clearance, V_z/F apparent terminal volume of distribution, *LLOQ* lower limit of quantification, *NC* not calculated (inadequate data to estimate parameter), *ND* not determined

[a] μg eq.h/mL for total radioactivity

[b] *n*=4

[c] *n*=2 (for the other patients too few data points were above the limit of quantitation to estimate an terminal disposition rate constant, therefore these parameters could not be calculated)



◀ **Fig. 3** Mean (+ standard deviation) cumulative percent of dose recovery of total radioactivity (*TRA*) in urine, feces and in total (urine and feces combined) (**a**), of TRA, lenvatinib, M1, M2, M3 and M5 in feces (**b**) and of TRA, lenvatinib, M1, M2, M3 and M5 in urine (**c**), following a single oral administration of 24 mg (100 μCi) ^{14}C-lenvatinib

The recovery of unchanged lenvatinib accounted for approximately 2.5 % of the administered dose and M2 for approximately 5 %. The total recovery of lenvatinib, M1, M2, M3 and M5 combined comprised less than 8 % of the administered dose.

Safety and efficacy

The mean duration of lenvatinib treatment was 152.8 days (range 42 to 279 days). The mean number of cycles received was 5.83, with a range of 2 to 10. Dose reductions due to treatment emergent adverse events (TEAEs) were applied for five patients.

During the mass balance phase, there were no serious adverse events or Grade 3 or above TEAEs. During the extension phase, five serious adverse events (other than death) were reported in two patients (33 %). Grade 3 or above TEAEs were reported in 3 patients (50 %). All 6 patients experienced at least one TEAE that was considered treatment-related. TEAEs considered probably related to the lenvatinib treatment were stomatitis in four patients (67 %), diarrhea and dysphonia in three patients (33 %), dry skin in two patients (33 %), and constipation, glossodynia, nausea, oral pain, vomiting, fatigue, oral candidiasis, blood pressure increase, weight decrease, proteinuria and hypertension in one patient (17 %).

Two deaths occurred in the serious adverse event follow-up period of the extension phase, a 30-day period post study drug administration. Both deaths were due to disease progression and considered unrelated to the study drug.

From start to the end of the study, no clinically important changes in mean hematology, biochemistry and urinalysis values were noticed and no clinically significant changes in mean vital signs and ECGs were observed.

The efficacy of lenvatinib treatment, in terms of best overall tumor response as assessed by the investigator, was partial response in one patient (17 %) and stable disease in three patients (50 %). Two patients (33 %) had progressive disease.

Discussion

In this study, radiolabeled lenvatinib was used to investigate the pharmacokinetics and excretion of lenvatinib in humans. Secondary objectives were to assess the safety and to explore the antitumor effect of lenvatinib as a single daily dose of 24 mg.

Table 2 Cumulative recovery of the dose in urine, feces and in total (urine and feces combined) (n=6), based on total radioactivity measurements and calculated for lenvatinib, M1, M2, M3 and M5 based on LC-MS/MS measurements following a single oral administration of 24 mg (100 μCi) ^{14}C-lenvatinib (n=6)

Matrix	Cumulative dose recovery – geometric mean, % (CV, %)					
	TRA	lenvatinib	M1	M2	M3	M5
Urine	24.7 (17.8)	0.636 (95.6)	0.030 (119)	0.133 (60.4)	0.025 (115)	0.014 (196)
Feces	63.6 (11.2)	1.86 (59.0)	0.025 (77.9)	4.76 (54.3)	NC	0.130 (30.8)
Total	88.6 (10.4)	2.52 (67.7)	0.056 (77.3)	4.90 (53.6)	0.025 (115)	0.146 (18.7)

Percent dose recovery is cumulated until the end of the collection period, which is 168 h post-dose for urine and 240 h post-dose for feces

TRA total radioactivity, *NC* not calculated, concentrations in all samples were <LLOQ

The short time to peak plasma concentration (1.6 h) indicates rapid absorption of lenvatinib and is consistent with previous studies, wherein the maximum lenvatinib plasma concentrations were typically observed between 1 and 3 h post-dose [9, 15]. Also the subsequent rapid decline and the final slower decline with an elimination half-life of 34.5 h is in line with previous studies [9]. Lenvatinib's rapid absorption, long terminal elimination half-life and large apparent volume of distribution (V_z/F=336 L), are similar to those of other tyrosine kinase inhibitors [3, 16]. The lower percentage of lenvatinib and total radioactivity in whole blood as compared to plasma (29 % and 36 % lower, respectively) suggest that lenvatinib and lenvatinib-related products are less well distributed into red blood cells.

The half-life based on the plasma total radioactivity measurements was smaller than the half-life of lenvatinib in plasma. This can be explained by the difference in LLOQ, which was 13–14 ng eq/mL for total radioactivity measurements and 0.25 ng/mL for unchanged lenvatinib in plasma. While total radioactivity was quantifiable up to 24–96 h post-dose, unchanged lenvatinib could be measured up to 144–168 h post-dose, allowing a more accurate determination of half-life. For a similar reason, the pharmacokinetic parameters of lenvatinib based on whole blood concentrations are likely less accurate than those obtained with plasma concentrations. Although the LLOQ of lenvatinib was the same in whole blood and plasma, the sampling for plasma was more frequent than for blood. This may partly explain the high oral and renal clearance and the low apparent terminal volume of distribution and half-life calculated based on blood concentrations as compared to the values obtained with plasma concentrations.

Metabolism is important in the elimination of lenvatinib. Unchanged lenvatinib comprised 60 % of the exposure to total radioactivity in plasma, indicating the presence of other lenvatinib-related products. Because reference standards were available for lenvatinib metabolites formed by decyclopropylation (M1), demethylation (M2), N-oxidation (M3) and *O*-dearylation (M5), these metabolites were quantitatively assessed in all samples. It appeared however that their contribution to the total elimination of lenvatinib was limited. Measurable plasma concentrations of these metabolites were only found for M2 and M5 and maximum concentrations were at least 700 times lower than the lenvatinib peak plasma concentration. Therefore, other lenvatinib metabolites are expected to be present in plasma.

The excretion of lenvatinib and its metabolites occurred mainly via the fecal route (Fig. 4). Lenvatinib metabolites other than M1, M2, M3 and M5 are also expected in urine and feces, since the recovery of lenvatinib, M1, M2, M3 and M5 in urine and feces combined accounted for less than 8 % of the administered dose. Additionally, as Fig. 3c shows, there is a continued excretion of total radioactivity in urine when the excretion of the quantified metabolites have already reached their maximum (around 24 h), suggesting the formation of downstream metabolites. The identification of the additional

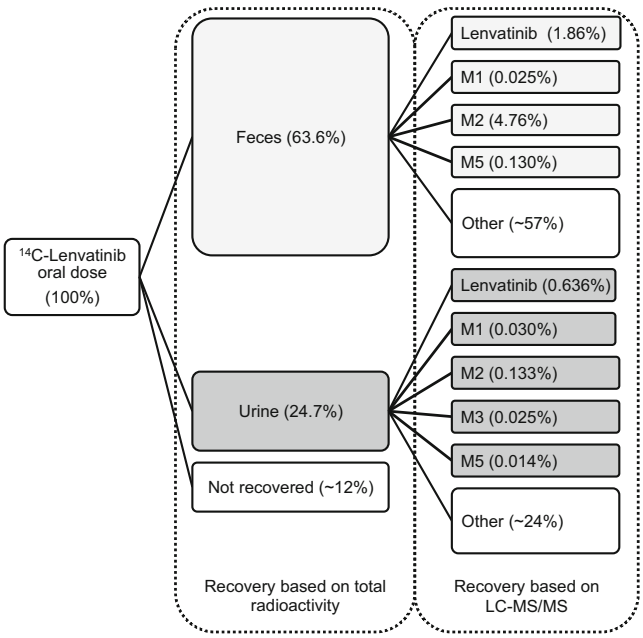


Fig. 4 Overview of the average mass balance of ^{14}C-lenvatinib in 6 cancer patients following a single oral administration of 24 mg (100 μCi) ^{14}C-lenvatinib. Values are geometric means and percent dose recovery is at the end of the collection period, which is 168 h post-dose for urine and 240 h post-dose for feces

lenvatinib metabolites in excreta and plasma is subject of further investigation.

The minor contribution of lenvatinib to the total of excreted lenvatinib-related compounds (Fig. 4) suggests that both the kidney and the liver play an important role in the metabolism of lenvatinib. This mass balance study therefore warrants to further examine the effect of renal and hepatic impairment on the pharmacokinetics of lenvatinib, and clinical trials to investigate this have been performed. The results of a hepatic impairment trial were recently published by Shumaker et al. and led to the conclusion that a reduced dose is advisable for subjects with severe hepatic impairment [17].

Adverse events observed in this study that were considered probably treatment-related were mainly of gastrointestinal origin and were comparable with side effects observed for other tyrosine kinase inhibitors [3]. Although designed as a secondary objective, antitumor activity of lenvatinib may have been observed, since partial response was observed in one and stable disease in three of the total six patients, as the best overall tumor response. The efficacy of lenvatinib is being further explored in Phase II and Phase III trials.

In conclusion, this study showed that lenvatinib is rapidly and well absorbed and extensively metabolized, with subsequent excretion in urine and, more predominantly, in feces. In addition, lenvatinib showed acceptable safety and preliminary antitumor activity.

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Conflict of interest A.C. Dubbelman, H. Rosing, C. Nijenhuis, A.D.R. Huitema, M. Mergui-Roelvink, J. H.M. Schellens and J.H. Beijnen declare they have no conflict of interest. A. Gupta is employee of Eisai Ltd., D. Verbel and R. Shumaker are employees of Eisai Inc. G.A. Thompson is a paid consultant to Eisai Inc.

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