PET imaging of zirconium-89 labelled cetuximab

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Phase I trial

PET imaging of zirconium-89 labelled cetuximab: A phase I trial in patients with head and neck and lung cancer

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ABSTRACT

Background and purpose: PET imaging of cetuximab uptake may help selecting cancer patients with the highest chance of benefit. The aim of this phase I trial was to determine the safety of the tracer ^{89}Zr-cetuximab and to assess tumour uptake.

Methods: Two dose schedules were used; two consecutive doses of 60 MBq ^{89}Zr-cetuximab or a single dose of 120 MBq, both preceded by 400 mg/m^2 of unlabelled cetuximab. Toxicity (CTCAE 3.0) was scored twice weekly. PET-CT scans were acquired on days 4, 5 and 6 (step 1) or 5, 6, 7 (step 2). Because tumour uptake could not be assessed satisfactorily, a third step was added including EGFR overexpressing tumours.

Results: Nine patients were included (6 NSCLC; 3 HNC). No additional toxicity was associated with administration of ^{89}Zr-cetuximab compared to standard cetuximab. A tumour to blood ratio (TBR) > 1 was observed in all but one patient, with a maximum of 4.56. TBR was not different between dose schedules. There was a trend for higher TBR at intervals > 5 days after injection.

Conclusions: Both presented ^{89}Zr-cetuximab administration schedules are safe. The recommended dose for future trials is 60 MBq, with a minimum time interval for scanning of 6 days.

Monoclonal antibodies are increasingly used in anticancer treatment to specifically target receptors at the surface of tumour cells, either as a monotherapy or in combination with radiotherapy or chemotherapy. Cetuximab is such a monoclonal antibody that specifically blocks the epidermal growth factor receptor (EGFR) which is over-expressed in many human malignancies [1,2]. EGFR activation and overexpression appear to be important tumour cell mechanisms in the development of resistance to radiation and chemotherapy, resulting in decreased rates of local tumour control and survival [3].

A randomized phase III trial in head and neck cancer showed improved survival of adding cetuximab to radiotherapy [4]. In contrast, cetuximab combined with radiotherapy failed to show any benefit over chemoradiotherapy [5], moreover, the addition of cetuximab to chemoradiotherapy did not show benefit in head and neck cancer [6] or non-small cell lung cancer [7,8]. Selection of patients with the highest chance of benefit from cetuximab treatment is of obvious relevance, also in view of its high costs. The mechanisms underlying clinical response or resistance to treatment against EGFR with cetuximab combined with radiotherapy are, however, largely unknown [9,10].

Non-invasive visualization and quantification of tumour uptake of cetuximab may contribute significantly to the selection of patients and determination of the needed dosage [11]; several methods using nuclear imaging have been proposed [12,13].

As the biologic half-life of cetuximab in blood is 65–95 h [14], a radioactive tracer with a long half-life is needed to visualize its uptake. Zirconium-89 (^{89}Zr), with a half-life of approximately 78 h, is an example of such a positron emission tomography (PET) tracer that can successfully be labelled to cetuximab and has shown promising results in animal models [15–17]. In a

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preclinical study, uptake of ^{89}Zr-cetuximab was demonstrated only in EGFR-positive tumours. However, it was shown that ^{89}Zr-cetuximab uptake did not correlate with EGFR expression levels, implying that pharmacokinetic and -dynamic factors influence the cetuximab accumulation in tumours [16].

Here, we report the results of a phase I study with primary aim determining the safety of zirconium-89 labelled cetuximab, with tumour ^{89}Zr-cetuximab uptake as a secondary end point. Furthermore, we aimed to get an indication of the optimal radioactivity dose and imaging time point to direct future phase II studies.

Methods

Patient selection

Patients with a histologically confirmed solid cancer without curative treatment options were eligible. Inclusion criteria were: WHO performance status 0–2; adequate bone marrow, hepatic and renal function; life expectancy > 3 months and written informed consent. Patients with a recent (<3 months) myocardial infarction, uncontrolled infectious disease, pregnancy, previous administration of cetuximab or concurrent treatment with anticancer agents or radiotherapy were excluded.

Study design

A study design with two different dose schedules was used (Fig. 1). In both steps, ^{89}Zr-cetuximab was administered within one hour after administration of the loading dose of unlabelled cetuximab. Six patients were included, three in each dose schedule. If in any patient grade 2 or higher toxicity was observed related to ^{89}Zr-cetuximab administration, 3 extra patients would be included in this dose step. When at maximum 1/6 patients experienced grade 2 toxicity, the step was considered safe.

The study design anticipated the future aim, which is to determine the tumour uptake of ^{89}Zr-cetuximab before and during therapy. Therefore, in the first dose schedule (step 1), toxicity of two consecutive low doses of ^{89}Zr-cetuximab was investigated. A standard loading dose of 400 mg/m^2 cetuximab, followed by 10 mg of ^{89}Zr-cetuximab (60 MBq) was administered on day 0. A second injection with a maintenance dose of 250 mg/m^2 of cetuximab, followed by 10 mg of ^{89}Zr-cetuximab (60 MBq) was administered on day 14.

As a larger radioactivity dose of ^{89}Zr-cetuximab is possibly needed to obtain the best image quality, toxicity of a single larger dose was investigated in a second dose schedule (step 2). A loading dose of 400 mg/m^2 of cetuximab was administered followed by injection of 10 mg of cetuximab labelled with 120 MBq ^{89}Zr.

During inclusion in step 2, a study amendment was written based on the first results of the image analysis. It was decided that if the secondary endpoint (assessment of tumour ^{89}Zr-cetuximab uptake) could not be satisfactorily assessed based on the first 6 patients, a third step would be added in which 3 patients were included with an EGFR overexpressing tumour based on recent immunohistochemistry. For these patients, the administration schedule was the same as in step 2.

The study protocol was approved by the medical ethical committee and the radiation safety committee. The study is listed in clinicaltrials.gov number NCT00691548.

EGFR expression and mutation status

In all patients, biopsies of the primary tumour were taken before the start of treatment. For patients included in step 3, a recent biopsy of the primary tumour or from a metastatic lesion had to be available, without any antitumour treatment between the biopsy and inclusion. After inclusion, EGFR expression and mutation status (exons 18–21) as well as *KRAS* mutation (codons 12 and 13) were assessed on these biopsies [18]. EGFR expression was analysed with the EGFR pharmDx qualitative immunohistochemical kit system (Novocastra and Dako, Denmark). To quantify EGFR expression, both EGFR membrane staining intensity and the percentage of EGFR expressing cells were taken into account. EGFR membrane staining intensity was expressed as a score between 0 (none) and 3+(strong). The percentage of cells staining at different intensities was assessed visually. Subsequently, EGFR immunohistochemistry (IHC) scores were defined on a scale of 0–300 using a formula combining percentage of staining cells and staining intensity [19]. Tumours were divided in showing low and high EGFR-expression on basis of a score <200 and ⩾200, a threshold based on the results of the FLEX study [20,21].

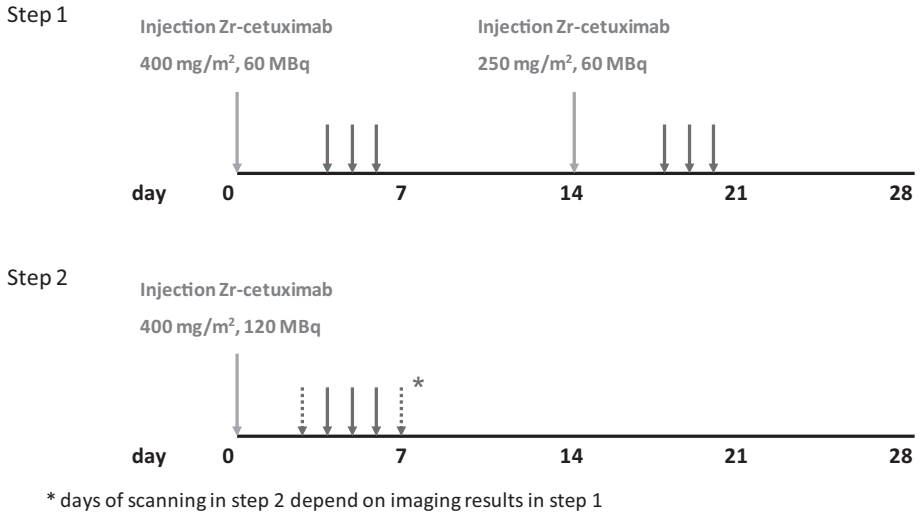

Fig. 1. Timeline of the study: ^{89}Zr-cetuximab injection (grey arrows) and the acquiring of ^{89}Zr-cetuximab PET/CT scans (black arrows). In step 2, PET-CT scans on days 3 and 7 after injection were optional and depended on the imaging results in step 1: If in step 1 two or more patients had a higher tumour to blood ratio (TBR) on day 4 than on day 5, an additional scan on day 3 would be performed. If in step 1 two or more patients had a higher TBR on day 6 than on day 5, an additional scan on day 7 would be performed. Patients included in step 3 were scanned at one time point only, based on the imaging results of steps 1 and 2.

Toxicity scoring

Toxicity was assessed during treatment and twice weekly thereafter until 14 days after the last injection, according to the CTCAE 3.0 scoring system. At baseline and on days 7 and 14 after injection, blood testing was performed for haematology, kidney and liver function.

The most common side effect of cetuximab administration that was anticipated for was skin toxicity (acneform rash) [22]. As skin toxicity is a known side effect of unlabelled cetuximab administration and this trial aimed to investigate the safety of labelled cetuximab, skin toxicity was not regarded as a dose limiting side effect.

Synthesis of ^{89}Zr-cetuximab

^{89}Zr was produced by a (p,n) reaction on natural ^{89}Y as described by Verel et al. [23]. Subsequently, labelling of ^{89}Zr to the mAb cetuximab was performed as previously reported [24].

PET-CT imaging

PET-CT imaging was performed at days 4, 5 and 6 after injection in dose step 1. Imaging intervals in step 2 were adapted based on the TBRs at subsequent days in step 1. Patients included in step 3 were scanned at one time point only, based on the imaging results of steps 1 and 2. Furthermore, an optional ^{18}F-fluorodeoxyglucose (FDG-)PET scan was performed within the study period, at least 24 h before or 12 days after ^{89}Zr-cetuximab administration ($\geqslant$4 times the half-life of cetuximab). In the first patient that underwent an FDG-PET scan after the ^{89}Zr-cetuximab administration, an extra PET-CT scan was performed at day 12, preceding FDG-injection, to assess remaining activity associated with the ^{89}Zr -cetuximab administration.

Image analysis

Tumour sites and normal tissues were manually delineated on one of the ^{89}Zr-cetuximab PET-CT scans (day 6 or 7) by the same observer (JvL). For one patient, delineations were performed on the FDG-PET-CT scan. Tumour sites were delineated based on the CT using FDG-PET information when available. To quantify uptake in muscle and liver, a transversal CT slice of the subscapular muscle and the liver was delineated. The resulting regions of interest were subsequently projected onto the other ^{89}Zr-cetuximab-PET-CT scans and FDG-PET scan through co-registration of the corresponding CT images using rigid registration.

The mean, maximum and peak standardized uptake value (SUV_{mean}, SUV_{max} and SUV_{peak}, respectively) were determined using in-house developed dedicated software. The tumour SUV_{peak} was defined by calculating the mean SUV in a sphere with a diameter of 1.2 cm within the tumour region with the highest activity. Tumour to background ratio (TBR) was calculated by dividing tumour SUV_{peak} by SUV_{mean} in the aortic arch.

For patients in whom an additional ^{18}F-FDG-PET scan was performed, the ^{18}FDG and ^{89}Zr-cetuximab uptake was visually compared.

Results

Patient characteristics are shown in Supplementary Table 1, tumour characteristics in Table 1. Nine patients, with a median age of 62 years (range: 53–75), were included. The third patient was excluded from the study before injection of ^{89}Zr-labelled cetuximab, because of malignant hypertension during administration of the unlabelled cetuximab. The blood pressure normalized within two hours of observation without further

Table 1
Tumour characteristics and tumour and normal tissue uptake.

	Primary tumour				Schedule	FDG-PET	^{89}Zr-cetuximab PET							
						Tumour					Liver		Muscle	
	Site	Histology	Mutation*	EGFR IHC score		SUVmax	TBRpeak	SUVpeak	SUVmax	SUVmean	SUVmax	SUVmean	SUVmax	SUVmean
1	Lung	SCC	NA	NA	Step 1	11.26	2.78	4.74	7.05	1.80	NA	NA	3.41	0.59
2	Lung	LCC	k-ras: + EGFR: –	80	step 1	-	3.11	4.28	7.84	2.14	NA	NA	2.62	0.55
3**	Lung	LCC	-	-	Step 1	-	-	-	-	-	-	-		
4	Lung	AC	k-ras: – EGFR: –	280	Step 1	–	4.56	3.83	7.62	1.30	8.11	5.45	1.43	0.37
5	Lung	SCC	k-ras: – EGFR: –	240	Step 2	10.21	0.96	1.18	1.62	0.79	7.75	5.68	0.97	0.21
6	Lung	AC	k-ras: – EGFR: –	290	Step 2	7.58	1.74	3.59	6.62	1.85	9.92	9.23	1.27	0.32
7	Oropharynx	SCC	k-ras: – EGFR: –	300	Step 2	11.28	3.20	2.95	4.32	1.97	6.98	5.26	1.63	0.27
8	Oropharynx	MC	k-ras: – EGFR: –	270	Step 3	–	1.35	4.35	6.19	3.15	7.11	6.81	1.78	0.75
9	Oropharynx	SCC	k-ras: – EGFR: –	290	Step 3	–	1.46	4.77	6.92	2.95	18.42	12.47	1.24	0.44

SCC: squamous cell carcinoma; LCC: large cell carcinoma; AC: adenocarcinoma; MC: muco-epidermoid carcinoma; NA: not assessable due to insufficient quantity of histological material; TBR: tumour to blood ratio.

* k-ras: codons 12 and 13; EGFR: exons 18–21.

** Excluded patient.

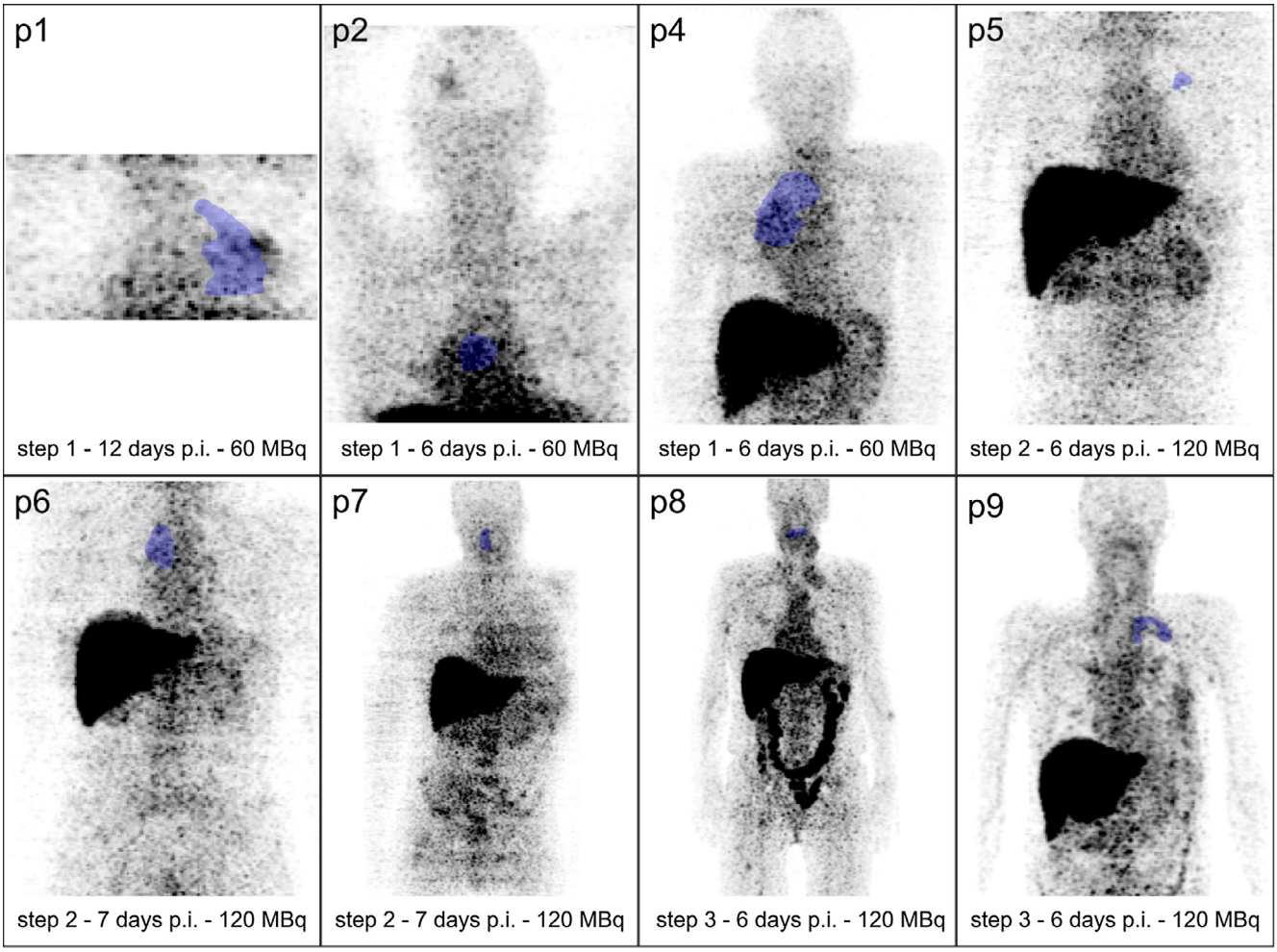

Fig. 2. ^{89}Zr-cetuximab PET maximum intensity projections of all patients. The GTV of the primary tumour is overlaid in blue. The notes underneath the images indicate in which dose step the patients were included, how many days post injection the displayed images were acquired and the dose of ^{89}Zr-cetuximab (2 × 60 MBq or 1 × 120 MBq) administered. The scans with the highest TBR in the primary tumour were selected. For dose step 1, the images after the first injection were analysed.

consequences. Therefore an extra patient (patient 4) was included in dose step 1. Only 2 patients were included in step 3 of the study. Given the slow accrual, and since this step was not necessary to reach the primary endpoint, it was decided to close this step prematurely.

From the 9 patients included, 6 had non-small cell lung cancer (NSCLC) and 3 had head and neck cancer (HNC). All patients had previously undergone anti-cancer therapy. In one patient, EGFR expression and mutation and *KRAS* mutation status of the primary tumour could not be assessed due to insufficient quantity of histological material. In the other patients, 6 of the 7 primary tumours showed high EGFR expression, while none showed a mutation of the EGFR gene. An example of IHC staining in a biopsy with high EGFR expression is shown in Supplementary Fig. 1 (Patient 6). In the only patient with low EGFR expression, the primary tumour showed a mutation in the *KRAS* gene. One of the two patients included in step 3 showed high EGFR expression on a recent tumour biopsy. The recent specimen of the second patient contained insufficient material to allow a reliable EGFR analysis.

Each patient received ^{89}Zr-cetuximab administration as planned. Patients included in steps 1 and 2 underwent the ^{89}Zr-PET-CT scans at 3 consecutive days, except for patient 6 who could not undergo the first scan due to pain. Patient 4, included in step 1, was scanned at day 5–7 instead of day 4–6 because of logistical reasons. In two patients (patient 2 and 4) enrolled in the first dose step, the series of PET-CT scans after the second injection could not be performed due to pain and dyspnoea. Patients included in step 3 were scanned at day 6 after injection. Four patients underwent an FDG-PET scan within the study period, at an interval of 12 or 13 days after the injection of ^{89}Zr-cetuximab.

An overview of the toxicity per patient is presented in Supplementary Table 2. No toxicity other than skin rash was observed in any of the patients. Of the eight patients that received the full cetuximab administration, three experienced grade 1, and 4 experienced grade 2 acne form rash. No changes were observed for haematological, kidney and liver function compared to baseline.

^{89}Zr-cetuximab-PET images of all patients are shown in Fig. 2 (coronal) and Fig. 3 (transversal). Tumour and normal tissue uptake values of ^{89}Zr-cetuximab are shown in Table 1. All but one patient had a peak TBR > 1 at any of the imaging time points. For each patient, the ^{89}Zr-cetuximab image with the highest TBR was selected for further analyses. For patient in dose step 1, only the scans after the first ^{89}Zr-cetuximab injection were analysed since for two of the three patients PET/CT could not be acquired in the second week. The average peak TBR was 2.39 (range 0.96–4.56). The average tumour SUV_{max} and SUV_{mean} were 6.02 (range: 1.62–7.84) and 1.99 (range 0.79–3.15), respectively. The average SUV_{max} and SUV_{mean} for the liver were 9.71 (range: 6.98–18.42) and 7.48 (range 5.26–12.47). For muscle, the average SUV_{max} and SUV_{mean} were 1.79 (range: 0.97–3.41) and 0.44 (range

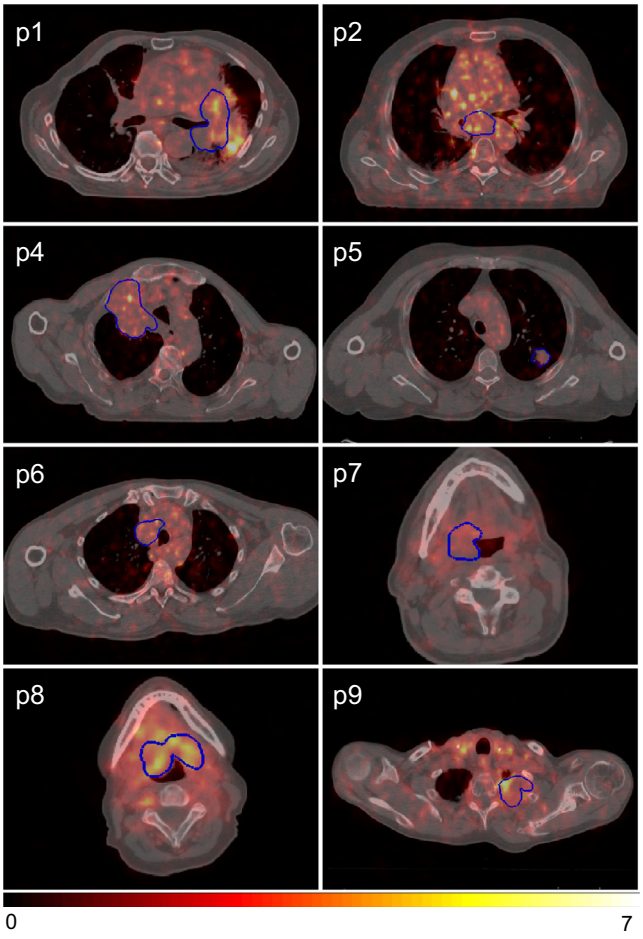

Fig. 3. ^{89}Zr-cetuximab PET-CT images at tumour level showing uptake in SUV. The primary tumour is delineated in blue. The scans with the highest TBR in the primary tumour were selected.

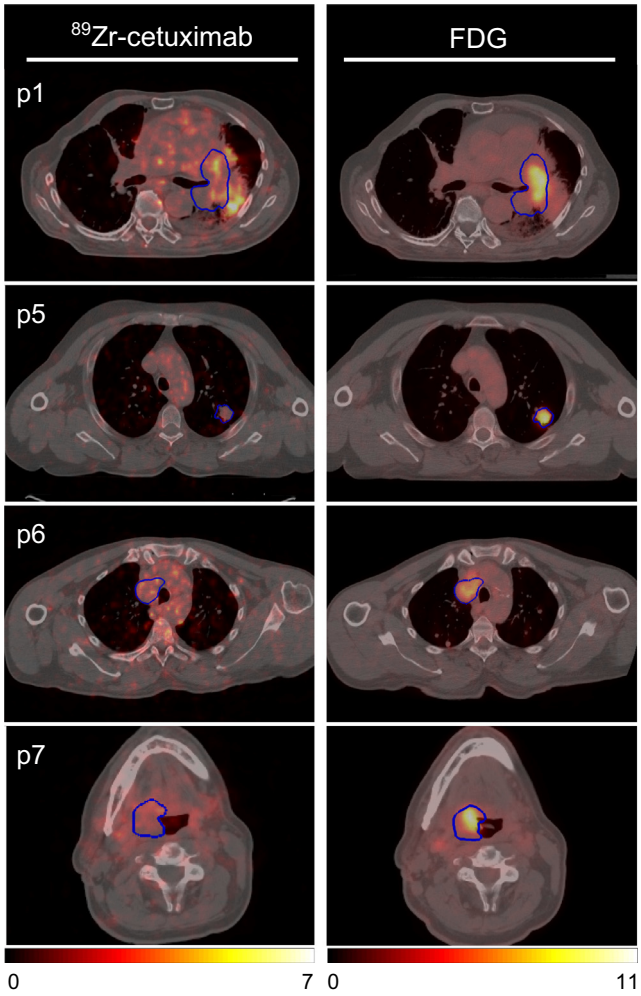

Fig. 4. Correlation between ^{89}Zr-cetuximab and ^{18}FDG uptake for the four patients receiving both scans. PET uptake is shown in SUV. The primary tumour is delineated in blue. The ^{89}Zr-cetuximab scans with the highest TBR in the primary tumour were selected.

0.21–0.75). Both SUV_{max} and SUV_{mean} of the tumour were significantly higher than the muscle SUV_{max} and mean.

The ^{18}FDG-PET and ^{89}Zr-cetuximab uptake of the four patients who had an FDG-PET-CT scan is shown in Fig. 4. Visual comparison showed a remarkable mismatch between FDG and ^{89}Zr-cetuximab uptake in one patient (patient 1, Fig. 4A).

No direct relationship was observed between the EGFR IHC score and TBR.

For the patients in dose step 1, the average peak TBR at days 4, 5 and 6 after the first injection was 1.33 (range: 1.23–1.43), 2.06 (range: 1.67–2.65) and 3.12 (range: 1.68–4.56), respectively (Fig. 5). As the highest TBR was found at day 6 after injection, imaging in dose step 2 was performed at days 5, 6 and 7. For patients in dose step 2, the average peak TBR at days 5, 6 and 7 after injection was 1.65 (range: 0.94–2.37), 1.64 (range: 0.96–2.71) and 1.96 (range: 0.94–3.20) (Fig. 5). The peak TBR of patients included in step 3 was 1.35 and 1.46 (day 6). At the regular imaging time points, the highest peak TBR was seen in patient 4 (4.56), at day 6 after injection. In patient 1, an extra PET-CT scan was performed at day 12 after injection. The TBR at this time point was higher than the maximum TBR for this patient at the regular imaging time points (2.78 and 1.68, respectively).

Discussion

The current phase I trial is the first study in HNC and NSCLC evaluating the safety of ^{89}Zr-cetuximab. It is a first step towards a new patient selection method for cetuximab treatment or the addition of cetuximab to radiotherapy. ^{89}Zr-cetuximab has until now only been evaluated in human patients with colorectal cancer [25]. In this study, no additional toxicity was associated with ^{89}Zr-cetuximab administration. Acne form eruption $\leqslant$ grade 2 was observed in 88% of patients, a rate comparable to that found with therapeutic administration of cetuximab [4,22,26]. One patient developed malignant hypertension during the administration of unlabelled cetuximab. Although rare, this side-effect has been described previously [27].

Tumour uptake of ^{89}Zr-cetuximab

It should be emphasized that no definitive conclusions can be drawn given the small amount of patients as well as the heterogeneity in tumour type and previous anticancer treatment. All but one patient that completed the study protocol showed a peak TBR > 1. Visually, however, the ^{89}Zr-cetuximab images showed a rather patchy distribution, without an evident specific uptake of ^{89}Zr-cetuximab within the tumour. Visually comparing the ^{89}Zr-cetuximab images of NSCLC and HNC patients, more discernible tumour uptake appeared to be present in at least one of the HNC patients (Fig. 3G).

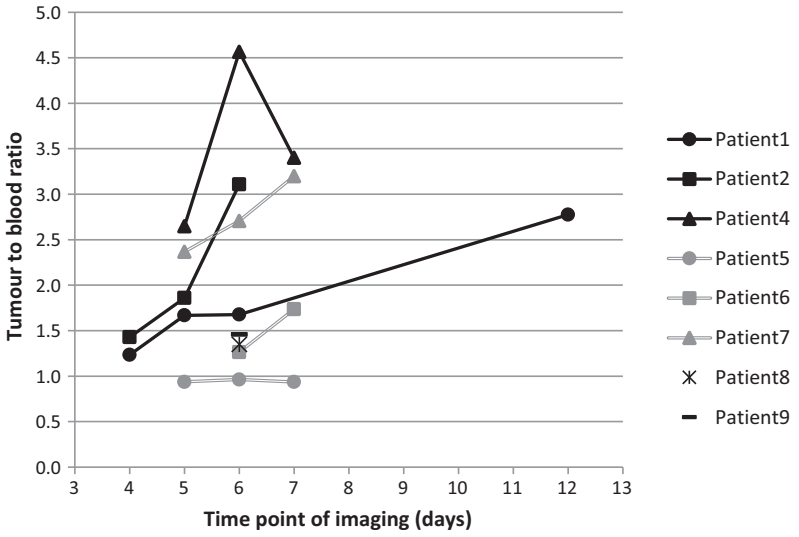

Fig. 5. ^{89}Zr-cetuximab tumour to blood ratios at different time points after injection of the tracer. The patients displayed with a black line were included in dose step 1; the patients with a grey line in dose step 2; the patients with a single black marker in dose step 3.

No direct relationship was observed between EGFR IHC score and TBR, similar to the findings in our preclinical study [16]. There are several considerations that argue a direct relationship between EGFR expression at a biopsy specimen and response to cetuximab treatment. Firstly as expression of EGFR can be induced by radiation and chemotherapy, it is possible that an initially EGFR negative tumour may become positive early during therapy, thereby still benefiting from cetuximab treatment [28,29]. Secondly, EGFR expressing tumours will not respond to cetuximab when cetuximab does not reach therapeutic concentrations in the tumour because of e.g. interstitial pressure and vascular perfusion changes [30].

Recommended ^{89}Zr-cetuximab dose and time interval for scanning

A large divergence was observed in the time curves of the TBR. Hence, no optimal imaging time point can be derived from these data. However, we advise a time delay of at least 6 days after injection, as 5 of the 6 patients that were scanned at days 5 and 6 showed a higher TBR at day 6. This is in line with a study in colorectal cancer that also found an optimal scanning time point 6 days post injection [25]. In the only patient that was scanned after a substantially longer time interval (12 days after administration), the highest TBR was found at this time point. This late rise in TBR suggests that a later imaging time point might result in more optimal tumour visualization. Due to decay, however, accurate quantification at such late time intervals may be biased by increased noise levels.

Although no definitive conclusions can be drawn regarding the dosage of labelled cetuximab in view of the different tumour characteristics in the two dose schedules, the current results do not indicate that a higher dose would result in more optimal TBRs. Therefore, taking into account the ALARA (as low as reasonably achievable) principle, a dose of 60 MBq of ^{89}Zr-cetuximab is recommended for future study.

Recommendations for further study

There are some other factors of which the influence on tumour visualization should be assessed in future studies. First, the loading dose administered in this study (400 mg/m^2), which is the standard therapeutic regimen, might be suboptimal to enable adequate tumour visualization. A loading dose of cetuximab is required as cetuximab first binds irreversibly to EGFR expressing liver cells, and tumour cells are only targeted after the liver has been saturated. This rationale is supported by animal studies as well as by the positive relation between skin toxicity and tumour response to cetuximab, implying that skin saturation only occurs after the liver has been saturated [31,32]. The liver SUV_{max} and SUV_{mean} in the current trial were rather high, implying that EGF receptors of the liver were still not saturated by the loading dose.

Based on the promising clinical results of adding cetuximab to radiotherapy in head and neck cancer [4], and the imaging data from the current study suggesting specific ^{89}Zr-cetuximab uptake in at least one of the HNC patients, a clinical trial was started in this patient group (e.g. in the ARTFORCE head and neck trial, clinicaltrials.gov: NCT01504815 [33]). Unfortunately, due to slow accrual, complexity of ^{89}Zr-cetuximab imaging for both patients and radiotherapy departments, and discontinuation of the funding of cetuximab, the study was amended and ^{89}Zr-cetuximab imaging and cetuximab treatment were discontinued.

In conclusion, the administration of ^{89}Zr-cetuximab to image *in vivo* cetuximab uptake is safe and not associated with any additional toxicity compared to unlabelled cetuximab. The recommended time interval between cetuximab administration and scanning is at least 6 days. The recommended ^{89}Zr-cetuximab dose is 60 MBq. Further research is needed to explore the optimal loading dose of non-labelled cetuximab and the relationship with EGFR expression.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.radonc.2016.11.020.

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