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PET imaging of hypoxia

Exploratory prospective trial of hypoxia-specific PET imaging during radiochemotherapy in patients with locally advanced head-and-neck cancer

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ABSTRACT

Purpose: To explore in a prospective trial the prognostic value of hypoxia imaging before and during radiochemotherapy in patients with locally advanced head and neck cancer.
Patients and methods: Twenty-five patients with stage III/IV head and neck cancer were investigated with [18F]-fluoromisonidazole (FMISO) PET/CT at four time points during radiochemotherapy (baseline, 8–10 Gy, 18–20 Gy,50–60 Gy). FMISO PET/CT image parameters were extracted including maximum-tumour-to-background (TBR_{max}) and thresholded volume at different TBR ratios. CT volume and baseline FDG-PET/CT image parameters were also included. Parameters at all time points were investigated for their prognostic value with the local-progression-free-survival endpoint (LPFS). Significance was evaluated with multivariate Cox (including clinical parameters) and Log-rank tests.
Results: FMISO-image parameters were found to have a strong association with the LPFS endpoint, and were strongest at the week 1 and 2 time points ($p = 0.023–0.048$ and 0.042–0.061 respectively on multivariate Cox). Parameters measured at baseline were only significant on univariate analysis. None of the clinical parameters, and also FDG- or CT-delineated volumes, were significantly associated with LPFS.
Conclusion: This prospective, exploratory study demonstrated that FMISO-PET/CT imaging during the initial phase of treatment carries strong prognostic value. FMISO-PET/CT imaging at 1 or 2 weeks during treatment could be promising way to select patients that would benefit from hypoxia modification or dose-escalated treatment. A validation study is on-going.

Hypoxia is characteristic of solid tumours, influences malignancy and contributes to treatment failure (Chapters 15 and 16, [36]). A recent meta-analysis from randomised trials demonstrated that hypoxia modification, such as use of carbogen inhalation and nimorazole [17], or nimorazole alone [26], improves local tumour control, disease-specific and overall survival [24]. Grouping patients according to hypoxic status, in the ARCON, TASMAN 92.02 and DAHANCA 5 studies, showed that patients with hypoxic tumours yield a much greater benefit from these treatments [16,29,35]. Furthermore, in a handful of imaging studies, hypoxia PET/CT imaging, as a non-invasive alternative to electrode measurements, has been shown to provide prognostic information [7,8,11,18,21,27,28]. Thus, while these imaging studies were small, hypoxia-tracer-PET/CT appears to be a promising method for assessing hypoxia-related tumour resistance, and, furthermore, may be of use to select patients that may benefit from hypoxia modification or intensified radiation therapy treatments.

The extent of tumour hypoxia and the capacity for reoxygenation during radiotherapy vary amongst patients. If tumours partially or fully reoxygenate during treatment, then a single pre-treatment measurement of hypoxia is unlikely to be a meaningful parameter for patient stratification. Nevertheless, pre-treatment PET/CT signal from hypoxia PET-tracers has been found in several studies to be prognostic for response to radiotherapy in the head and neck [8,11,18,21,27,29,34]. A study from Leuven in 15 patients performed [18F]-fluoromisonidazole positron emission tomography (FMISO PET/CT) imaging at baseline and during radiotherapy [9], finding both maximum tumour-to-blood ratio (T/B) at baseline and after 30 Gy prognostic for disease-free survival. A similar study in 14 patients from Tübingen found no prognostic significance of FMISO PET/CT during treatment, but was able to show that the hypoxia decreased in most patients during treatment [10].

Our hypothesis is that the individual dynamics of reoxygenation during radiotherapy in each head and neck cancer will play a role in the overall tumour response, with tumours that reoxygenate early during therapy having a more favourable prognosis. It is then

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Table 1
Patient and treatment characteristics ($n = 25$).

Characteristic	Value
Age (mean, range)	59 years (45–76)
Gender (female/male)	3/22
Primary site	
Oral cavity/oropharynx/hypopharynx/larynx	6/9/7/3
T-stage (T3/T4)	9/16
N-stage (N0/N1/N2a/N2b/N2c/N3)	5/2/0/3/12/2
Stage (III/IV)	3/22
$GTV_{primary}$* (median, range)	45 cm^3 (7–308)
p16 status (positive/negative/not available)	7/14/4
Radiation dose (mean, range)	72 Gy (69–72)
Overall treatment time (mean, range)	41 days (39–46)
Chemotherapy	
Cisplatin/5-Fu (patients, mean number of cycles, range)	21/6 (3–6)
Mitomycin C/5-Fu (patients, mean number of cycles, range)	4/2 (2–2)

* Defined by CT.

important to establish the optimal time point for performing hypoxia PET/CT imaging to stratify patients for more aggressive treatments. Here we report results from a hypothesis-generating study of sequential FMISO PET/CT imaging in head and neck cancer patients undergoing radiochemotherapy. The general approach of our prospective imaging trial, accruing 50 patients, is to collect imaging data into an exploratory and a validation cohort, with 25 patients per cohort. While trial is still on-going, we report here the results from our exploratory cohort, where four imaging time points are tested for their prognostic value. We also investigate which FMISO PET/CT image parameter is strongest for assessment of hypoxia with PET/CT, e.g. hypoxic volume – measured from a given tumour-to-background ratio (TBR) threshold, or maximum signal intensity.

Patients and methods

Patients

The imaging trial, recruiting up to 50 patients, was split into an exploratory and a validation cohort. A total of 28 patients were recruited between July 2006 and July 2009. Three patients withdrew from their imaging appointments during treatment, leaving a total of 25 patients that are included in this exploratory cohort analysis. The characteristics of the remaining 25 patients are summarised in Table 1. The study (NCT00180180) was approved by the German Federal Radiation Protection Authority (Bundesamt für Strahlenschutz) and the local Ethics Committee. Accrual of a second cohort with 25 planned patients is currently on-going. Inclusion criteria included written informed consent, age older than 18 years, histologically-proven squamous cell carcinoma of the head and neck that was not operated (for various oncological and non-oncological reasons), WHO performance status 0–2 and tumour location and extent suitable for radiotherapy with curative intent. The exclusion criteria were presence of distant metastases, previous radiotherapy in the head and neck region, previous history of malignant disease (other than T1 skin cancer or CIS of the uterine cervix), pregnancy and lacking capacity to consent.

Work-up, treatment and follow-up

The staging procedures included clinical examination, endoscopy, ultrasound, contrast-enhanced CT or MRI and FDG PET/CT scans. For imaging and treatment the patients' head and shoulders were immobilised in the supine position using a thermoplastic mask. Using all available information on disease localisation and extent, the gross tumour volume (GTV) and subsequently clinical target volume as well as planning target volume were defined in the CT-component of the baseline FDG-PET/CT. Treatment planning with 3D-conformal or intensity-modulated radiotherapy (Helax TMS 6.1A.1, since September 2008 Oncentra Masterplan 3.1, Nucleotron) aimed for uniform coverage of the PTV (95–107% of the prescribed dose), while aiming to maintain dose to the spinal cord ($D_{maz} < 45$ Gy) and sparing the larynx ($D_{mean} < 45$ Gy) and parotid glands ($D_{mean} < 26$ Gy). Doses of 72 Gy in 6 weeks were prescribed to the primary tumour and involved lymph nodes (PTV1) and of 59.4 Gy in 5 weeks to the lymphatic regions at risk for microscopic disease (PTV2). The first 30 Gy were given with conventional fractionation followed by 1.4 Gy twice daily for the remainder of the treatment. This followed a hyperfractionated, accelerated fractionation regimen that has been validated in a randomised trial, and is frequently used in Germany [5]. In one patient conventional fractionation with 70 Gy in 7 weeks (PTV1) and 50 Gy in 5 weeks (PTV2) was given. Concurrent chemotherapy consisted of 5-fluorouracil (continuous infusion, 120 h, 600 mg/m^2/day on days 1–5) and weekly cisplatin (30 mg/m^2) or mitomycin C (10 mg/m^2, days 5 and 36)[13]. Patients were followed-up 4 weeks after the end of treatment, then every 3 months for 2 years and at a 6-month frequency thereafter. Follow-up visits included clinical examination and alternating FDG-PET/CT or MRI.

PET/CT imaging

PET data acquisition was done using a Biograph 16 W PET/CT (Siemens Medical Solutions Inc., Knoxville, TN, USA) containing a 16-slice CT scanner. The FMISO-PET tracer was delivered from the Institute of Radiopharmacy at the Helmholtz Centre in Dresden-Rossendorf, and was synthesised as previously published[33].The imaging schedule included four time points as follows: before RCT, after a mean dose of 8–10 Gy (end of week 1), of 18–20 Gy (end of week 2) and of 50–60 Gy (during week 5) during RCT. Most patients (19/25) attended all four imaging time points. However, due to logistic reasons in six patients imaging at 10 Gy or 20 Gy was not possible. Similarly, it was not possible to image at the same day during the course of RCT in every patient. The pre-therapeutic FMISO-PET imaging was acquired 2–4 days after FDG-PET/CT imaging.

Static FMISO images were acquired at both 2 h and 4 h post injection (p.i.) after administration of 250–300 MBq. For each dataset one field of view (FOV; 16 cm in z-axis) was scanned for 15 min as a three-dimensional emission scan. The acquisition time, for the emission only, was 12 min. Emission images with CT-based attenuation-correction were reconstructed using four iterations, eight subsets and a 1 mm 3D Gaussian filter.

Before RCT an initial diagnostic whole body FDG-PET/CT, 60 min p.i. of 350 MBq [^{18}F]-FDG (GlucoRos®, Helmholtz Centre Dresden-Rossendorf, Germany), was acquired in every patient for staging. CT scans for attenuation correction, as well as for electron density determination, were performed in cranio-caudal direction from skull base to upper thighs. Scanning parameters included 100 mAs, 120 kV, online tube current modulation, 1.5 mm slice collimation, 0.5–0.75 s rotation time, and reconstruction of 5 mm slices. For diagnostic purposes and radiotherapy planning, contrast-enhanced CT scans were performed in most patients, using 120 ml of iodinated contrast material (Ultravist® 370, Bayer Schering Pharma, Leverkusen, Germany). The acquisition time per bed position for the emission FDG-PET scan was 3 min. Uncorrected emission images and CT-based attenuation-corrected images were reconstructed, both using four iterations, eight subsets and a 5 mm 3D Gaussian filter.

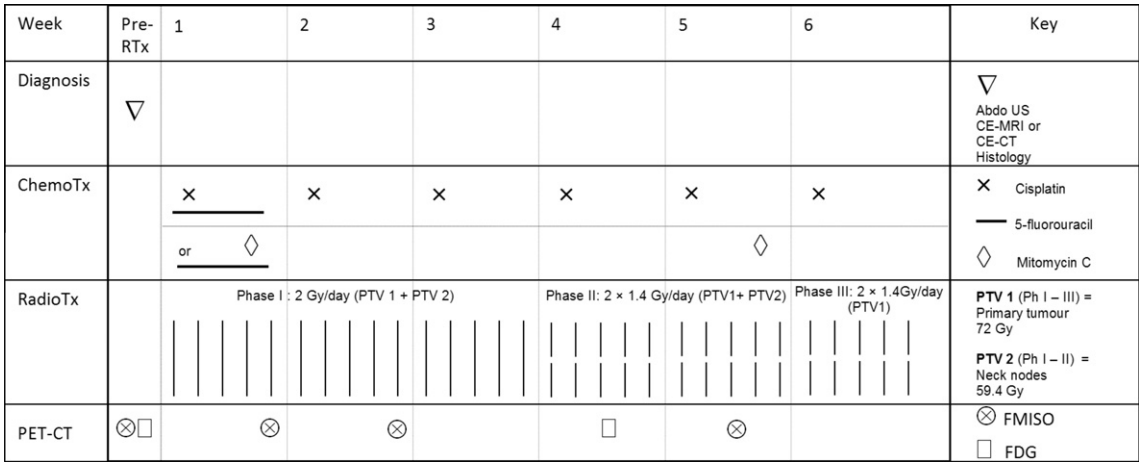

Fig. 1. Diagram of treatment and imaging workflow for the study.

At each imaging session patients were immobilised and positioned as for treatment. This ensured reproducible positioning, not only during radiation, but also during consecutive FMISO- and FDG-PET/CT.

Image analysis and data evaluation

PET/CT scans from different time points before and during treatment were imported into ROVER image analysis program (ABX, Radeberg, Germany). The scan set included the FDG- and the FMISO-PET/CT images taken before treatment commencement and the three FMISO-PET/CT images taken during treatment (see Fig. 1 for imaging schedule). The FMISO images selected for the analysis were those obtained 4 h p.i., since it was found in an earlier study from our institution that the 4 h images have superior contrast [1].

Each image in this scan set was co-registered to the pre-therapeutic CT (from the baseline FDG-PET/CT scan) using the CT-component of the FMISO-PET/CT scans using a rigid-registration algorithm (mutual information). This was possible since the patient had been set-up in each PET/CT scan in the same position using the radiotherapy mask. Weight loss in some patients caused the registration to be less straight-forward. In these cases, the registration was performed manually by viewing the anatomy in the vicinity of the tumour, and prioritising this region for matching. All registered scan sets were checked for matching precision by an experienced radiologist (NA). In a first step, an ellipsoidal region was drawn around the metabolically active tumour on the coregistered FDG-PET scan by visual inspection based on the apparent voxel signal. It included the primary tumour while excluding involved lymph nodes. This volume-of-interest (VOI) was used subsequently to mask the coregistered FMISO PET images. This was important so that measurement of the FMISO avid region did not include involved lymph nodes and regions of uptake in normal tissue.

A tumour-to-background-based thresholding technique was used to generate VOIs encompassing the regions of increased activity concentration on the FMISO-PET scans, or the hypoxic volume (HV). This was done step-wise as follows. The background measurement was obtained by generating ellipsoidal VOIs of approximately 20 cm^3 (or ~200 PET voxels) in the deep-neck muscle in each FMISO scan. The CT-component of the PET/CT scans was used to guide delineation of the neck muscle excluding bone and larger amounts of fat. The mean voxel value within this neck muscle VOI was used for the background measurement (B). HV was volume-thresholded at a range of tumour-to-background threshold levels (TBR) after a given dose (D), $HV_{TBR,D}$ and location of this centre of the HV was measured. Four TBR threshold levels ([1.4, 1.6, 1.8, 2.0] × B) were applied so that the most appropriate threshold level could be evaluated. Other potentially relevant parameters were also obtained. These included the baseline fractional hypoxic volume (as a fraction of CT-delineated tumour volume at baseline, HF_{TBR}), maximum TBR and maximum SUV in the volume after dose, D, ($TBR_{max,\ D}$, $SUV_{max,\ D}$), the metabolic tumour volume (MTV) segmented on FDG-PET/CT, and maximum SUV from the baseline FDG-PET ($SUV_{max,\ fdg}$). The segmentation algorithm used for the MTV was a background-subtraction adaptive-thresholding technique available within the ROVER software [14].The maximum TBR and SUV were determined by finding the mean value within the highest-valued voxels representing 0.5 cm^3 of tissue (this coincided approximately with the mean of the values above the 95th percentile).

To test the strength of each imaging parameter univariate and tentative (due to the low patient number) multivariate analyses were performed using the Cox proportional hazards model. Multi-variate analysis was performed to test the strength of each image parameters against clinical parameters T, N and HPV status. Log-rank tests were also performed stratifying the patients by the median of the image parameter. Kaplan–Meier curves were plotted for patient groups by the median TBR_{max} for all imaging time points.

Results

Median follow-up of all patients was 12 months (range 2–58). At time of analysis nine patients are alive with a median follow-up of 32 months (range 20–58). Five patients died from intercurrent disease without evidence of disease progression within the first 6 months during follow-up. Local failures were observed in eight patients resulting in a local recurrence free survival at 3 years of 57%. Among them one patient showed in addition a nodal failure. In one patient a distant metastasis was detected at 5 months following treatment, and then, 7 months later, a local failure was detected.

Twenty-one patients out of 25 (84%) had a hypoxic volume at baseline ($HV_{1.6,\ 0Gy} > 1\ cm^3$ or 13 PET voxels). Median fractional hypoxic volume (HF) at baseline, using TBR = 1.4, 1.6, 1.8 and 2.0 for thresholding, was 0.66, 0.26, 0.15 and 0.04 respectively. Median hypoxic volume (thresholded at all TBRs) and median TBR_{max} decreased as treatment progressed (see Table 2). A hypoxic subvolume ($HV_{1.6,\ 57Gy} > 1cm^3$) remained in 10/24 patients in week 5 of treatment. The FDG volume of the primary tumour at baseline

Table 2
Hypoxia imaging parameters before and during radiochemotherapy. Data represent median and range. Hypoxic volumes (HV) were calculated using 1.4, 1.6, 1.8 and 2.0 as threshold relative to the background. TBR_{max} represents the maximum ratio between FMISO up-take in the tumour and the background, i.e. in the deep neck muscles.

Parameter	Baseline (0 Gy)	Week 1 (8–10 Gy)	Week 2 (18–20 Gy)	Week 5 (51–57 Gy)
$HV_{1.4}$ (cm^3)	31.5 (0.4–155.1)	28.2 (0–143.1)	21.8 (0–115.6)	2.0 (0–48.4)
$HV_{1.6}$ (cm^3)	13.2 (0–101.0)	11.6 (0–99.2)	6.0 (0–69.5)	0.3 (0–10.2)
$HV_{1.8}$ (cm^3)	5.8 (0–70.8)	3.1 (0–63.1)	0.4 (0–38.3)	0 (0–5.69)
$HV_{2.0}$ (cm^3)	2.0 (0–59.8)	1.0 (0–34.1)	0.1 (0–28.2)	0 (0–1.1)
TBR_{max}	2.2 (1.4–4.0)	2.2(2.0–3.9)	1.9 (1.7–3.4)	1.7 (1.5–2.2)
SUV_{max}	2.6 (1.9–4.3)	2.5 (1.7–4.3)	2.2 (1.0–3.8)	1.7 (0.3–2.4)

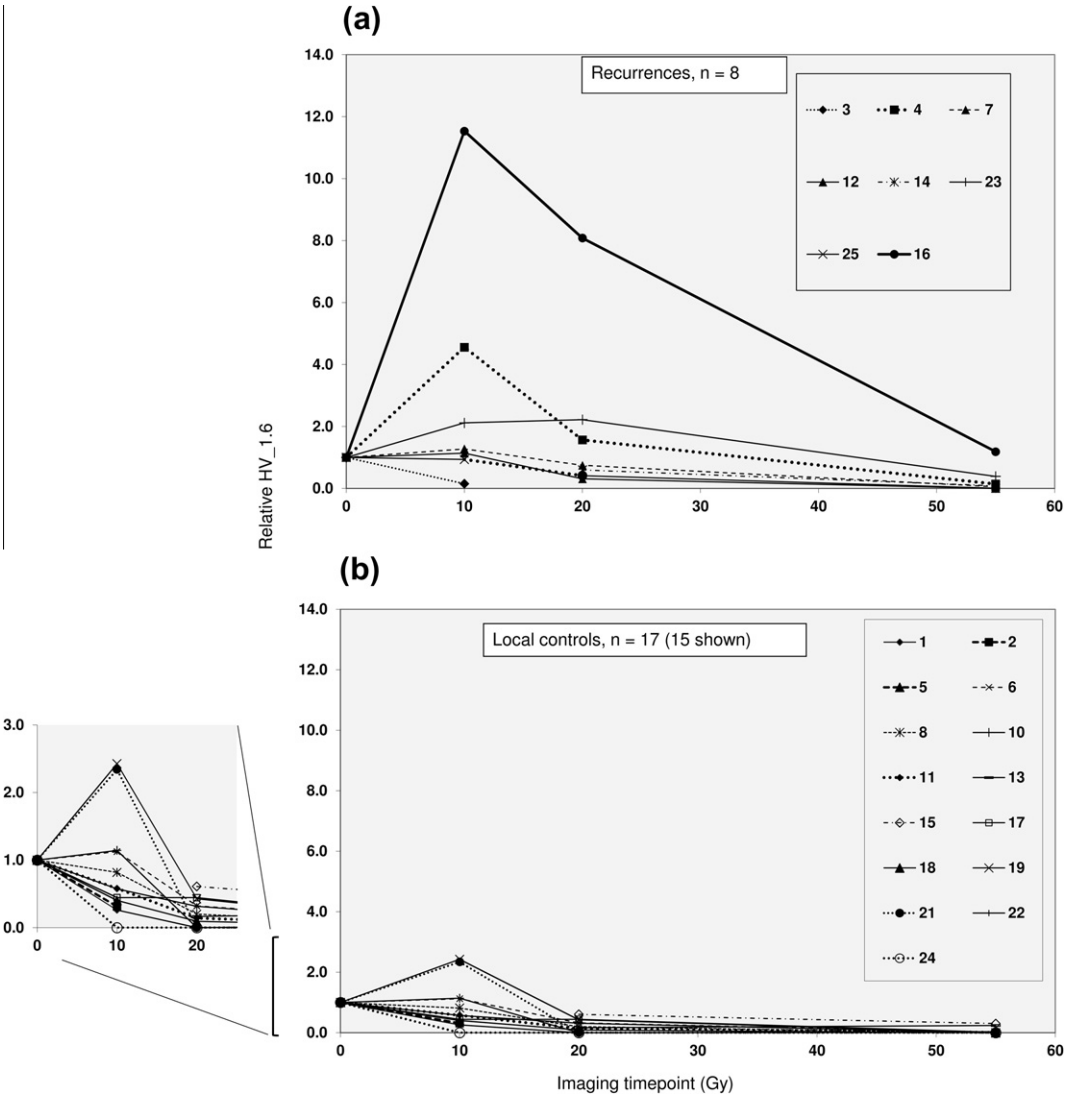

Fig. 2. Plots showing reoxygenation behaviour of tumours that (a) recurred and (b) were controlled. HV_1.6 denotes hypoxic volume generated from thresholding at a level of 1.6 times background. Relative HV_1.6 was relative to the baseline volume thresholded for each patient.

(thresholded at 40% of maximum) ranged between 4 and 136 cm^3, and correlated strongly with the volume manually-delineated on the contrast-enhanced CT (R^2 = 0.88). CT volume correlated significantly with baseline hypoxic volumes $HV_{1.4,\ 0Gy}$ and $HV_{1.6,\ 0Gy}$, but not with $HV_{1.8,\ 0Gy}$ and $HV_{2.0,\ 0Gy}$ (Pearson's correlation coefficients of 0.585, 0.383, 0.321, 0.265 respectively).

A range of behaviours in tumour reoxygenation were found. To demonstrate this the $HV_{1.6}$ relative to baseline for each patient over the four imaging time points are plotted in Fig. 2, both for responders and non-responders. There were tumours that showed only a very small hypoxic subvolume at baseline, and then exhibited large hypoxic volumes at the 10 and 20 Gy time points. Tumours with hypoxic volumes that did not resolve at 20 Gy (to below the baseline-measured volume) 3/3 recurred. Others had a progressive decrease in hypoxic volume after the start of treatment or after the 10 Gy time point.

On Cox analysis, none of the clinical parameters were significantly associated with local recurrence. Neither the FDG- or CT-delineated volumes nor HPV status was significantly associated with local recurrence on uni- or multivariate analysis (p = 0.34,

1.0 and 0.24 respectively). The hypoxic volume (using all thresholds) and TBR_{max} were most significantly associated with local recurrence at the 8–10 Gy and 18–20 Gy time points on both uni- and multi-variate Cox analyses (see Table 3). Image parameters measured at the baseline and the week 5 imaging time points were only significant on univariate Cox analysis. The Hazard Ratio (HR) of the hypoxic volume on univariate Cox analysis increased significantly with the threshold level used at 18–20 Gy (1.03–1.14), while the $TBR_{max,\ 20Gy}$ carried a HR of 4.25. Comparing the *p*-values and the HRs of SUV_{max} and TBR_{max} on multivariate Cox analysis, TBR_{max} appeared to be a stronger parameter at baseline or 18–20 Gy. However, at 8–10 Gy they had comparable strength.

Log-rank tests, for groups above and below the median of each of the image parameters, revealed significant difference between the groups for $TBR_{max,\ 20Gy}$, $TBR_{max,\ 57Gy}$, $HV_{1.6,\ 10Gy}$, $HV_{1.6,\ 20Gy}$, $HV_{1.8,\ 20Gy}$, $HV_{2.0,\ 20Gy}$, $HV_{1.8,\ 57Gy}$, $HV_{2.0,\ 57Gy}$. The median values of all parameters at all-time points are given in Table 2. It can be seen that a TBR_{max} of around 2.0 was a strong cut-off point between high and low risk groups at the first three imaging time points (Fig. 3).

Discussion

In this prospective exploratory study we show that measurement of hypoxia with FMISO PET/CT is a promising biomarker to distinguish patients at increased risk from local recurrence. The study design included three imaging time points during radiochemotherapy, enabling the time course of reoxygenation to be studied.

A particularly intriguing result of our study was that at early imaging time points during treatment, image characteristics became stronger prognostic parameters (compared to baseline), and were significant on multivariate analysis, albeit with limited power due to the low number of patients. This result is in line with preclinical experiments at our centre, where it was found that tumour control probability in human head and neck cancer xenografts correlated with hypoxic fraction after 10 fractions of 2 Gy, but only weakly [38] or not at baseline [39].

Our findings at baseline on univariate analysis agreed with other studies where hypoxic-tracer-PET imaging at baseline was reported to have prognostic value [9,11,18,21,27,29]. However, our results showed that the baseline image parameters were not the strongest predictors of local recurrence, as evidenced by their lack of significance on multivariate analysis and in part in the log-rank tests. There are little data from hypoxia imaging during treatment. Dirix et al. [8] found that maximum tumour-to-blood ratio >1.17 during treatment correlated with disease-free survival in a smaller cohort of 15 patients, with seven recurrences. However this maximum tumour-to-blood cannot be directly compared to our values, since the earlier study used blood samples to measure the background activity concentration. A report of FMISO-PET/CT imaging from Lee et al. [20] found that only 2/16 oropharyngeal carcinoma patients had hypoxia at week 4 of therapy, and pre- or during treatment FMISO PET/CT imaging parameters had no prognostic value. Eschmann et al. [10] also performed FMISO PET/CT imaging during treatment. But this study did not attempt to draw correlations with treatment outcome due to the small sample size ($n = 14$). This highlights the fact that small study samples can result in varying results, which must in part be due to the

Table 3
Univariate and multivariate Cox model *p*-value for image parameters is also given for testing with nodal and HPV status. HR denotes Hazard Ratio obtained.

Time point	Parameter	HR univariate	*p*-Value	HR multivariate	*p*-Value
Baseline	Age	0.98	0.657	1.06	0.417
	T stage	0.79	0.753	0.48	0.337
	N stage	4.39	0.167	3.66	0.163
	p16 status	0.59	0.529	0.43	0.239
	$GTV_{primary}$ (CT)	1.00	0.468	1.00	0.579
	FDG SUV_{max}	0.97	0.651	0.97	0.750
	FDG Vol	1.01	0.208	1.01	0.335
	$HV_{1.4}$	1.03	0.053	1.05	0.212
	$HV_{1.6}$	1.04	0.018	1.07	0.117
	$HV_{1.8}$	1.05	0.028	1.08	0.154
	$HV_{2.0}$	1.06	0.025	1.13	0.151
	$HF_{1.4}$	1.53	0.402	1.60	0.424
	$HF_{1.6}$	3.52	0.346	11.46	0.193
	$HF_{1.8}$	17.66	0.090	16.13	0.162
	$HF_{2.0}$	176.7	0.032	179.8	0.148
	FMISO TBR_{max}	5.16	0.022	12.85	0.139
	FMISO SUV_{max}	2.35	0.139	1.77	0.444
Week 1 (8–10 Gy)	$HV_{1.4}$	1.02	0.047	1.02	0.068
	$HV_{1.6}$	1.02	0.047	1.04	0.033
	$HV_{1.8}$	1.03	0.040	1.07	0.023
	$HV_{2.0}$	1.06	0.024	1.12	0.020
	FMISO TBR_{max}	2.76	0.025	6.04	0.040
	FMISO SUV_{max}	2.72	0.024	3.79	0.060
Week 2 (18–20 Gy)	$HV_{1.4}$	1.02	0.035	1.05	0.042
	$HV_{1.6}$	1.03	0.021	1.08	0.045
	$HV_{1.8}$	1.07	0.011	1.12	0.042
	$HV_{2.0}$	1.14	0.013	1.62	0.047
	FMISO TBR_{max}	4.25	0.008	6.19	0.061
	FMISO SUV_{max}	3.85	0.015	3.72	0.169
Week 5 (51–57 Gy)	$HV_{1.4}$	1.03	0.212	1.04	0.237
	$HV_{1.6}$	1.14	0.183	1.33	0.129
	$HV_{1.8}$	1.18	0.029	6541	0.132
	$HV_{2.0}$	16.8	0.027	10,194	0.106
	FMISO TBR_{max}	20.1	0.048	10,088	0.059
	FMISO SUV_{max}	1.57	0.559	2.18	0.453

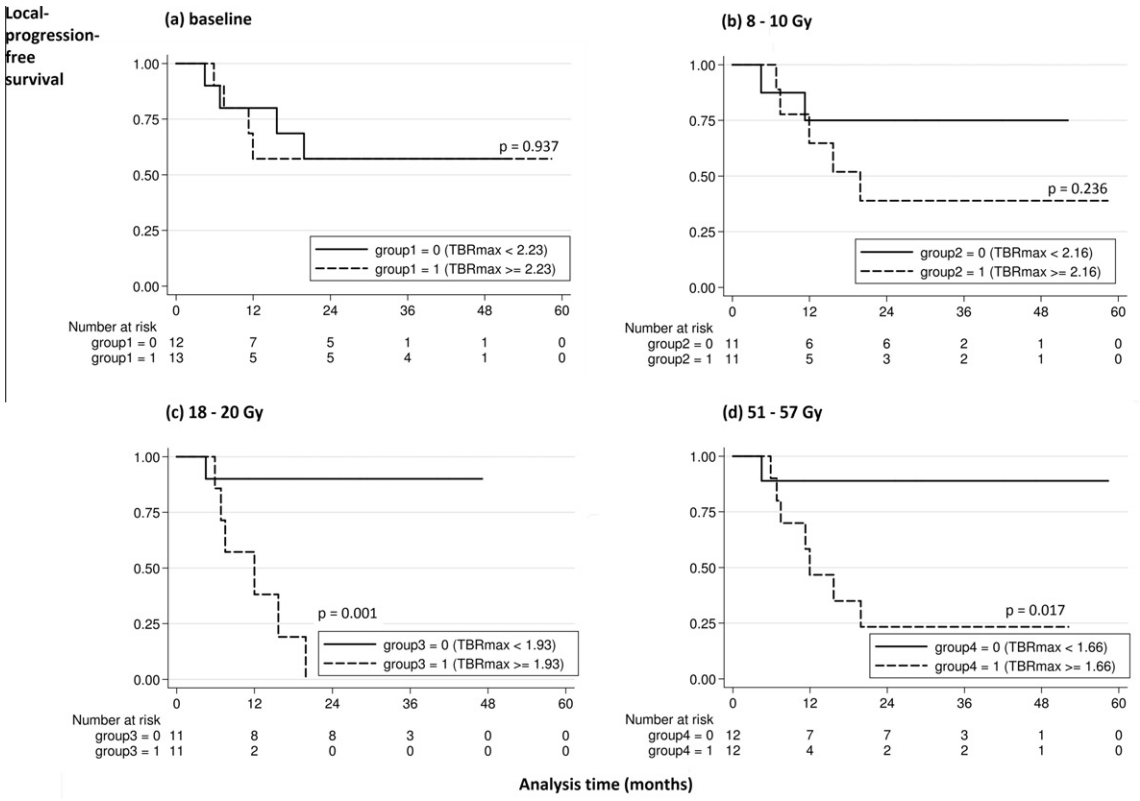

Fig. 3. Local-progression-free survival of patients stratified by the median value of the maximum tumour-to-background (TBR_{max}) at the given imaging time point.

large variation in radiation response that is known to exist in tumours [37]. Nevertheless, Horsman et al. recently published a meta-analysis that concurs with our study results, showing that hypoxia imaging with PET/CT has predictive value [15].

Different reoxygenation profiles of tumours may partly explain why hypoxia measurement during treatment was most optimal for prediction of local recurrence in our study. A range of behaviours in tumour reoxygenation was found, which was in line with the preclinical studies mentioned above and data published previously by our group [4] and previous accounts where hypoxia was measured in patients with Eppendorf electrodes [6,32]. An interesting finding was that some tumours having a small hypoxic subvolume at baseline then increased in size at the 10 Gy time point. Stadler et al. [32] also observed this behaviour in a small group of head and neck tumours. Apart from temporary impairment of perfusion with subsequent increase in hypoxia early during radiotherapy one may speculate about a different explanation. In FMISO PET/CT imaging the delivery of the tracer to the site of hypoxia is critical. In poorly perfused regions of tissue, which are by this very fact subject to hypoxia, tracer delivery and wash-out will be hampered, and thus the fraction of bound FMISO in a region may not reflect the underlying level of hypoxia. After several radiotherapy fractions it is conceivable that perfusion improves, leading to better tracer delivery and wash-out of unbound tracer. However, despite partial reperfusion of some regions, allowing for faster delivery and wash-out of the tracer, hypoxia may still remain in regions that are at a distance from the perfused vessels. In this case, this remaining hypoxia will be imaged with a higher fidelity. Modelling studies have indeed demonstrated that due to the tracer dynamics and vascular fraction a confounding picture on imaging may result [22]. To further explore this potential mechanism, perfusion measurements from dynamic-contrast-enhanced MRI [23] or CT [2] may be able to add complementary information to hypoxia imaging, and help to give a clearer picture of tumour microenvironment. Additionally, a less lipophilic tracer compound, such as [^{18}F]-FAZA, may improve tracer distribution in the tumour by facilitating faster wash-out of non-bound tracer [12,31].

The message coming out of hypoxia modification trials in head and neck is that patients should be selected for hypoxia modification based on hypoxia status of the tumour cancer (DAHANCA 5 [25], TASMAN 92.02 [29], ARCON [16]). The same should hold true for radiation dose-escalation strategies. Thus, deriving a reliable predictive biomarker from FMISO PET/CT imaging is extremely relevant. A convenient finding of the present study was the ability to select the high risk patients at early time points during treatment. This would be an advantage selection of patients for more aggressive treatment regimes, either hypoxic modification or radiation dose escalation. On the other hand, it may be advantageous to perform FMISO PET/CT later in treatment for the purposes of adaptive radiotherapy. Studies have shown significant tumour shrinkage in the head and neck during treatment, indicating some benefit from adapting radiotherapy treatment volumes [3,30]. This strategy could potentially maximise the boost dose prescription to high risk tumours while keeping within normal tissue tolerances.

Since the patient data from this study will be evaluated in two halves – an exploratory and a validation cohort – patient numbers in the present study were limited. Furthermore, missed imaging appointments in six patients (three patients at 10 Gy and three at 20 Gy) limited the statistical power. We propose this as a reason for which the N stage and HPV status were not found to be significantly associated with local recurrence, in disagreement with previous studies. There were also contradictory results found between Cox and log-rank tests at the baseline time point, where hypoxia imaging parameters were not significant on log-rank while being significant on univariate Cox analysis (see Fig. 3 for the K-M plot and Table 3). This could partially be explained by the fact that

the Cox model uses the parametric data, i.e. the magnitude of the imaging parameter of interest, while the log-rank tests groups above and below the median. Nevertheless, since the cohort was small, all statistical testing, especially multivariate analysis will be sensitive to outliers, and should be interpreted with caution. This will be addressed in the validation cohort and overall analysis of the whole data set and in a planned multi-institutional meta-analysis of individual patient data within the German Consortium for Translational Cancer Research (DKTK).

In order to find the hypoxic volume we used tumour-to-background-based segmentation techniques. While all due care was taken to ensure this method was consistent throughout the patient group, it was observed this method is sensitive to placement of the background ROI and signal noise within the background. In the study, the standard deviation of voxel values in the background ROI of 200 voxels was around 10%. This could lead to large uncertainties in volume thresholding, especially for lower contrasts encountered at the later treatment time points. However, the use of a large ROI was justified due to the large component of statistical noise inherent in low activity concentration regions, such as the background. The ROI size led to a lower standard error on the measurement given a high number of sampled voxels. Furthermore, segmentation of PET data based on voxel intensities, especially at low contrast-to-noise ratios, contains further sources of error, as carefully described by a recent publication [19]. The partial volume effect is known to confound quantitative information from PET images. For small tracer-avid volumes (<2 cm in diameter), the hypoxic volume will be underestimated due to poor recovery of the actual activity concentration in the voxels. Secondly, for physiological tracer accumulation, especially in hypoxia imaging where the ground truth is a pattern of ribbon-like accumulations on the micron scale, the image does not reflect the form of the hypoxic subvolumes. The signal arising from a voxel is rather a volume-average of hypoxic regions distributed within a voxel-sized volume of tissue. Thus, we acknowledge that hypoxic volume on thresholding can only be regarded as semi-quantitative. Currently, from the data available and the consideration given above, we would not propose that segmentation of FMISO-PET using thresholding could render the precise "high risk" subvolumes for dose-painted radiation therapy, especially at the currently available spatial resolution and contrast-to-noise ratio.

In conclusion, the present study explored the use of FMISO PET/CT before and during treatment as a way to select patients at high risk of developing local recurrence. We showed that FMISO imaging during the initial phase of treatment carries strong prognostic value for identifying patients at risk from local recurrence. On the other hand, baseline imaging was not found to be as strong in comparison, as different treatment-related reoxygenation profiles exist within individual tumours.

Overall, our exploratory study suggests that FMISO PET/CT imaging at 1 or 2 weeks during treatment is a promising way to select patients that may benefit from hypoxia modification or dose-escalated treatment. This finding needs further evaluation and validation in an independent data set, which is underway.

Conflict of interest statement

There are no conflicts of interest to declare.

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