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PET imaging of hypoxia

FAZA PET/CT hypoxia imaging in patients with squamous cell carcinoma of the head and neck treated with radiotherapy: Results from the DAHANCA 24 trial

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ABSTRACT

Purpose: Hypoxia is a cause of resistance to radiotherapy, especially in patients with head and neck squamous cell carcinoma (HNSCC). The purpose of this study was to evaluate ^{18}F-fluoroazomycin arabinoside (FAZA) positron emission tomography (PET)/computed tomography (CT) hypoxia imaging as a prognostic factor in HNSCC patients receiving radiotherapy.

Material and methods: Forty patients with HNSCC treated with radiotherapy (66–76 Gy) were included. Static FAZA PET/CT imaging 2 h post injection was conducted prior to irradiation. The hypoxic volume (HV) was delineated using a tumor-to-muscle value $\geqslant$1.4. In 13 patients, a repetitive FAZA PET/CT scan was conducted during the radiotherapy treatment.

Results: A hypoxic volume could be identified in 25 (63%) of the 40 tumors. FAZA PET HV varied considerably with a range from 0.0 to 30.9 (median: 0.3) cm^3. The T_{max}/M_{med} ranged from 1.1 to 2.9 (median: 1.5). The distribution of hypoxia among the Human Papillomavirus (HPV) positive (12/16) and negative (13/24) tumors was not significant different. In the FAZA PET/CT scans performed during radiotherapy, hypoxia could be detected in six of the 13 patients. For these six patients the location of HV remained stable in location during radiotherapy treatment, though the size of the HV decreased. In 30 patients a positive correlation was detected between maximum FAZA uptake in the primary tumor and the lymph node. During a median follow up of 19 months a significant difference in disease free survival rate with 93% for patients with non hypoxic tumors and 60% for patients with hypoxic tumors could be detected.

Conclusion: This study emphasizes the role of FAZA PET/CT imaging as a suitable assay with prognostic potential for detection of hypoxia in HNSCC.

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Hypoxic volumes exist in most solid tumors [1]. Hypoxia is a cause of resistance to radiotherapy; hence, it is an important prognostic factor, which is particularly well-documented in squamous cell carcinomas of the head and neck (HNSCC) [2]. Modification of tumor hypoxia improves loco-regional control and survival in patients with HNSCC treated with radiotherapy [3].

One approach to overcome hypoxia in tumors is to increase the radiation dose selectively to resistant volumes of the tumor by intensity modulated radiation therapy (IMRT), without increasing the dose to the normal tissue [4]. In standard radiotherapy treatment, the radiation dose prescribed is based upon morphological information. HNSCC are however, biologically highly heterogeneous tumors containing volumes of diverse radiation resistance. By integrating biological information it is possible to escalate the dose exclusively to the more resistant volumes of the tumor. This is expected to increase disease free survival by improving local tumor control without compromising the normal tissue. However, no clinical data have yet confirmed this concept. The search for reliable and clinical applicable methods to identify tumors that would benefit from hypoxia targeted therapy is ongoing.

PET imaging with a hypoxia specific tracer is a non-invasive method that provides a three-dimensional image of the degree of hypoxia within the tumor [5]. Consequently, PET imaging of tumor hypoxia is an obvious method to use for dose escalation of hypoxic regions within the tumor as PET scans can be repeated to detect spatial and temporal fluctuations in the hypoxic volumes [6].

Various hypoxia specific tracers have been tested clinically, mainly based on nitroimidazoles. Nitroimidazoles are reduced under hypoxia and become bound to cell components in hypoxic cells only [7]. The first tracer to be tested clinically was ^{18}F-flouromisonidazole (FMISO), which is still the most widely used [8–10]. The novel hypoxia specific tracer ^{18}F-fluoroazomycin arabinoside (FAZA) has generated higher tumor-to-background ratios com-

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pared to FMISO in preclinical studies [11,12]. This is due to a more rapid clearance of unbound tracer from non-hypoxic tissues [11], making FAZA a more attractive tracer for clinical use.

The DAHANCA 24 protocol was initiated with the primary aim to investigate the prognostic value of FAZA imaging in patients with HNSCC treated with radiotherapy.

Materials and methods

Patient and tumor data

Forty patients with newly diagnosed HNSCC referred for radiotherapy were prospectively included in the DAHANCA 24 protocol between December 2009 and October 2011. Eligibility criteria were: squamous cell carcinoma (T1-4 N0-N3 M0) of the larynx, pharynx or oral cavity; age above 17 years; no serious concurrent medical disease; no other current or prior malignant disease that could affect the treatment, evaluation or outcome of current disease; and the patient should be able to complete the treatment and follow-up. Patients were recruited at Aarhus University Hospital ($n = 27$) and Odense University Hospital ($n = 13$). Thirty-two males and eight females participated. Tumors were staged on the basis of clinical examination and relevant imaging modalities. Patients and tumor characteristics can be seen in Table 1.

The DAHANCA 24 study was approved by the local ethics committees according to Danish law and regulations. The trial was reported to Clinicaltrials.gov (ID: NCT01017224).

Treatment

Treatment was given with curative intent according to the DAHANCA guidelines (www.dahanca.dk). All forty patients received primary radiotherapy combined with the hypoxic cell sensitizer nimorazole (1200 mg/m^2), and in addition, 22 patients received weekly concomitant cisplatin (40 mg/m^2). No elective neck dissection was conducted.

Radiotherapy was prescribed in fractions of 2 Gy with a minimum dose of 66–68 Gy, 5–6 fractions per week during 5½–6½ weeks. Tumor size and location determined treatment dose. One patient (ID7) received accelerated hyperfractionated radiotherapy (76 Gy, in 56 fractions). All patients, except one (ID 9) received the planned treatment.

Follow up was conducted according to the DAHANCA guidelines with clinical examination at 2 months after completion of radiotherapy and then every 3 months during the first 2 years and every 6 months for the next 3 years. If a recurrence was suspected, confirmative CT or MRI scan was performed. Detailed description of the treatment is given in Supplementary Table 1.

Table 1
Patient and tumor characteristics as a function of FAZA hypoxia status.

Patient/tumor Data	All patients ($n = 40$)	Hypoxic[b] ($n = 25$)	Non hypoxic[b] ($n = 15$)	*P*
Age (years)				
Median	61	61	61	
Range	(44–80)	(44–80)	(48–77)	
<61 years	18	10	8	
⩾61 years	22	15	7	n.s.[a]
Gender				
Female	8	6	2	
Male	32	19	13	n.s.[a]
Tumor site				
Supraglottic larynx	9	4	5	
Hypopharynx	3	3	0	n.s.[a]
Oropharynx	25	15	10	
Rhinopharynx	3	3	0	
Tumor classification				
T1-2	28	14	14	
T3-4	12	11	1	**0.02**[a]
Nodal stage				
N0	10	4	6	
N1-3	30	21	9	n.s.[a]
Disease stage				
I–II	8	2	6	
III–IV	32	23	9	**0.04**[a]
Tumor volume (cm^3)				
Median	15.2	19.5	10.6	
Range	(1.8–143.7)	(4.8–143.7)	(1.8–36.6)	
>15 cm^3	21	15	6	
⩽15 cm^3	19	10	9	n.s.

The values are in bold as they are the only significant values.
n.s., not significant.
[a] Fisher's exact test for comparison between hypoxic and non hypoxic groups.
[b] Tumors were separated by FAZA HV.

FAZA PET/CT imaging protocol

FAZA was produced as described previously [13]. Twenty-seven patients were scanned on a Siemens Biograph Truepoint 64 PET/CT scanner (Siemens, Medical Systems) and for the remaining thirteen patients the FAZA imaging were obtained using a General Electric Discovery STE PET/CT scanner (GE Healthcare Technologies). The non-fasting patients received 365 (range 246–410) MBq FAZA and were scanned 123 (range 117–137) minutes post injection. All patients were scanned in one bed position (10 min) on a flat table top, positioned by laser, and wearing a rigid mask immobilizing head, neck, and shoulders. To minimize patient misalignment, the same procedures for securing the position of the patient were done when the patients had the contrast-enhanced CT of the head and neck (planning CT) for the radiation treatment planning. FAZA emission data were corrected for attenuation, scatter, and random coincidences following iteratively reconstruction. Calibrations with a FAZA filled NEMA phantom were conducted to ensure consistency of measurements between scanners.

The baseline FAZA PET/CT scan was conducted prior to the start of radiotherapy treatment (median interval 6, range 0–9 days) and for 13 patients a second FAZA PET/CT scan was conducted during the radiotherapy. None of the FAZA scans were used for the radiation treatment plan as evaluation was performed post treatment.

Image evaluation of FAZA

In all patients the primary tumor defined as gross tumor volume ($GTV_{planning}$) from the planning CT was delineated by experienced radiation oncologists and radiologists. In 27 cases tumor $GTV_{planning}$ was delineated in the program Eclipse (Varian) and in 13 cases the program Pinnacle (Philips) was used. The planning CT and the CT part of the FAZA scan were automatically deformable fused using SmartAdapt (Varian). $GTV_{planning}$ from the planning CT was copied to the FAZA PET/CT, generating a $GTV_{FAZA\text{-}T}$. In a similar manner, $GTV_{FAZA\text{-}L}$ of the previously delineated pathological lymph nodes was produced, selecting the lymph node with the highest uptake of FAZA. For the non-hypoxic reference tissue, a region of five consecutive slices was outlined in the neck muscle in the FAZA PET/CT scan equivalent to a volume of 1.2 cm^3 (mean). An in-house script (MATLAB 7.12, The MathWorks Inc., Natick, MA, 2012) was used to determine FAZA uptake in the GTVs and in the reference tissue. Uptake was assessed as Standardized Uptake Values (SUV). To verify the SUV values measured by MATLAB, the tumor SUV for two patients was also determined by Eclipse and no difference was found. Maximum, median, and mean SUV uptake of FAZA for the tumor and lymph node ($GTV_{FAZA\text{-}T}$ and $GTV_{FAZA\text{-}L}$) as well as mean and median FAZA uptake in the reference tissue was determined. From these values SUV_{max} in $GTV_{FAZA\text{-}T}$ divided by SUV_{med} in the muscle

(T_{max}/M) and SUV_{med} in GTV_{FAZA-T} divided by SUV_{med} in the muscle (T_{med}/M) were calculated. The procedure was similar for GTV_{FAZA-L}.

In order to define a hypoxic volume (HV) in the tumors all voxels within GTV_{FAZA-T} expressing a tumor-to-muscle value equal to or above 1.4 was determined, creating HV. For each patient a histogram of FAZA SUV in the muscle (reference tissue) was obtained. To compare these values each patient's muscle histogram was normalized by its own mean, resulting in all patients having a muscle mean of 1. Subsequently all the histograms were pooled creating one common muscle histogram. The standard deviation was 0.13, hence a T/M_{mean} of 1.4 (mean + three standard deviations) was set as the threshold. Consequently, more than 99% of the normalized muscle values were below 1.4 in this material, the details are described in Supplementary Fig. 1. In this study, hypoxic tumors were defined as tumors containing a hypoxic volume (HV), whereas non-hypoxic tumors did not contain a hypoxic volume. The procedure of defining a HV was similar for GTV_{FAZA-L}. Additionally, the fractional hypoxic volume for the primary tumor (FHV) was defined as the HV divided by the entire GTV_{FAZA-T}. The threshold for hypoxia was selected blinded for patient outcome.

HPV data and analysis

Tumor HPV status was determined by immunohistochemical detection of p16 which is a reliable surrogate marker for HPV infection in tumors [14]. Using antip16^{INK4A} antibody clone JC8, as described elsewhere [15], tumors were classified dichotomously as either p16 positive (strong, diffuse nuclear and cytoplasmic staining in more than 70% of carcinoma cells) or p16 negative.

15-Gene hypoxia classifier

All 40 primary tumors were classified according to the 15-gene hypoxia classifier [16]. This classifier has previously been able to identify patients who had a significant benefit of hypoxic modification with nimorazole [17].

Endpoints and statistics

The primary endpoint was loco-regional control after radiotherapy defined as complete and sustained disappearance of disease in the T- and N-site. Secondary endpoints were disease free survival, correlation of FAZA uptake in T-site and N-site, and correlation of FAZA uptake at baseline compared to FAZA uptake during radiotherapy. Time to event was estimated from the first day of radiotherapy. The closing date for analysis was September 15, 2012. The study was designed to include 40 patients. This number was based upon previous studies, with a similar patient population using the Eppendorf oxygen electrode or pimonidazole as prognostic hypoxic markers, which have shown statistic significant differences in loco-regional control between patients with HNSCC [18,19].

Patient and tumor characteristics were compared with the Fisher's exact test. FAZA PET characteristics were compared with the Mann–Whitney Wilcoxon test. Correlation between FAZA uptake in primary tumor and lymph node was tested using Spearman's rank correlation. Difference in outcome between the hypoxic and the non-hypoxic group was estimated by Kaplan–Meier curves and by log-rank test, stratified for HPV-status. *P*-values less than 0.05 were considered significant. Analyses were performed with the STATA statistical package, version 10 (STATA Corp, College Station, TX).

Table 2
Relationship between FAZA uptake and HPV status.

FAZA PET data	All patients (n = 40)	HPV negative (n = 24)	HPV positive (n = 16)	P^*
T_{max}/M				
Median	1.5	1.5	1.7	
Mean	1.7	1.7	1.8	n.s.
Range	(1.1–2.9)	(1.2–2.9)	(1.1–2.9)	
T_{med}/M				
Median	1.1	1.1	1.1	
Mean	1.1	1.1	1.1	n.s.
Range	(0.8–1.7)	(0.8–1.6)	(0.9–1.7)	
HV ⩾ 1.4 (cm^3)				
Median	0.3	0.2	1.9	
Mean	4.4	3.9	5.2	n.s.
Range	(0.0–30.9)	(0.0–30.9)	(0.0–30.8)	
FHV ⩾ 1.4 (%)				
Median	2.8	1.0	13.6	
Mean	15.9	13.7	19.1	n.s.
Range	(0–91.7)	(0–71.7)	(0–91.7)	

n.s., not significant.
* Mann–Whitney–Wilcoxon test for comparison between HPV-negative and HPV-positive groups.

Results

FAZA uptake in the primary tumor

FAZA PET median HV varied considerably among all 40 tumors with a range from 0.0 to 30.9 (median: 0.3) cm^3; the FHV was between 0 and 91.7 (median: 2.8)%. The T_{max}/M was between 1.1 and 2.9 (median 1.5) and the median T_{med}/M was 1.1 (range 0.8–1.7), as shown in Table 2. Fig. 1 shows the ranking of the 40 tumors according to the HV (ranking of the remaining quantification methods can be seen in Supplementary Fig. 2). However, the shared ranking within the 25 hypoxic tumors in FHV and T_{max}/M was different from the ranking when using HV. The ranking of T_{med}/M was considerably different from the other quantification methods, as some of the 25 hypoxic tumors (as defined by HV) had low values of T_{med}/M.

Comparison between FAZA uptake and tumor characteristics

A hypoxic volume could be identified in 25 (63%) of the 40 tumors (Supplementary Fig. 3), whereas in 15 of the tumors all tumor-to-muscle voxels were below 1.4. Patient and tumor charac-

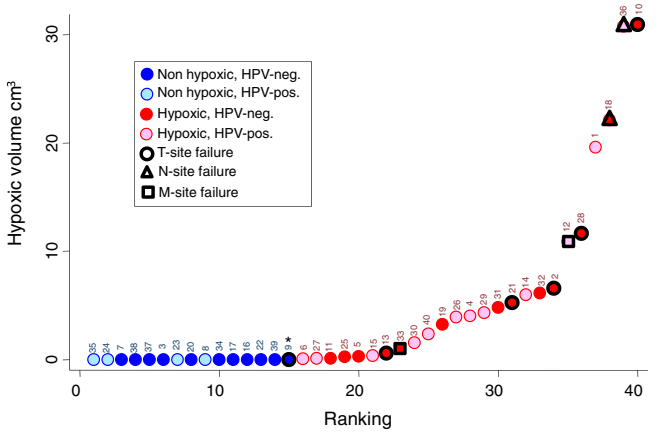

Fig. 1. Forty tumors ranked as a function of hypoxic volume. The hypoxic volume was defined by FAZA PET/CT imaging as all voxels within GTV_{FAZA-T} expressing a tumor-to-muscle value equal to or above 1.4. (*Patient ID 9 did not complete treatment).

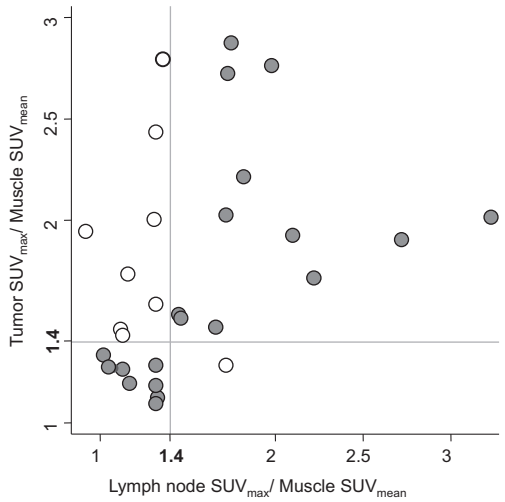

Fig. 2. Relationship between FAZA uptake in the primary tumor and the lymph node is shown as a scatter plot. The correlation is positive ($r = 0.517$; $P < 0.05$). The horizontal gray line shows the tumor-to-muscle threshold of 1.4 and the vertical line the similar lymph node-to-reference threshold. Dark points illustrate patients having a good correlation between the oxygenation status of the primary tumor and a lymph node, as both are either hypoxic or non-hypoxic. The white points illustrate discrepancy in the oxygenation status of the primary tumor and lymph node.

teristics as a function of FAZA hypoxia status can be seen in Table 1. There was no significant difference between the hypoxic and non-hypoxic tumors when accounted for age, gender, nodal stage, tumor volume or tumor site. A significant difference ($p = 0.02$) was found between T classification and FAZA hypoxic volume since more T3–T4 tumors were represented in the hypoxic group.

Relationship between FAZA uptake in primary tumor and lymph node

The relationship between FAZA uptake in the primary tumor and the related lymph node could be compared in 30 patients, as shown in Fig. 2. There was a positive correlation between maximum FAZA uptake in the primary tumor (T_{max}/M) and the lymph node of the same patient (L_{max}/M), Spearman $r = 0.517$; $p < 0.005$. However, when using the hypoxic volume to characterize tumors and lymph nodes into hypoxic versus non-hypoxic a difference was found in nine patients; eight patients had a hypoxic volume in the primary tumor, but no hypoxic volume in the lymph node and one patient (ID 26) had a hypoxic volume in a lymph node, but no hypoxia in the primary tumor as seen in Fig. 2 (since this patient have a hypoxic volume in the lymph node, but not in the primary tumor, the hypoxic lymph node is subsequently considered as GTV_{FAZA_T}). For 21 patients a match was found between the lymph node and primary tumor.

Repeated FAZA PET/CT imaging

Thirteen patients had two FAZA PET/CT scans separated by 14–28 (median: 14) days. Nine patients of the thirteen had a hypoxic volume at baseline. The median hypoxic volume for all thirteen patients was 0.3 (range 0–30.9) cm^3 and the median T_{max}/M was 1.6 (range 1.1–2.9). The majority of patients had no residual hypoxic volume at the second FAZA PET/CT scan; the range of the hypoxic volume was 0–20.2 (median 0) cm^3 and the median T_{max}/M was 1.4 (range 1.1–3.0). In six patients, hypoxic volumes could be detected at the second scan. Though the HV was smaller, the location remained stable in the tumors of these six patients, see Fig. 3. In these six patients four had treatment failure (including one patient with metastatic disease). Among the seven defined as having no residual hypoxia two treatment failures were found (one being the patient who did not complete treatment).

FAZA uptake and HPV

The HPV-positive and the HPV-negative tumors were found to be equally hypoxic (Fig. 3), as no significant difference was found in the distribution of T_{med}/M, T_{max}/M, HV or FHV (Mann–Whitney–Wilcoxon test), as seen in Table 2. When using the threshold of HV to distinguish between hypoxic/non-hypoxic tumors, 75% of the 16 HPV-positive tumors were hypoxic and 54% of the 24 HPV-negative tumors were hypoxic.

FAZA uptake and the 15-gene hypoxia classifier

The tumors were also ranked according to the expression of genes included in the 15-gene hypoxia classifier and separated in two groups with 14 (35%) more hypoxic and 26 (65%) less hypoxic. No correlation between hypoxic status, as assessed by the classifier and FAZA PET/CT imaging, was found (Supplementary Table 2).

FAZA PET and outcome

Complete remission was detected in thirty patients. Potential follow-up time, defined as the time elapsed between the first date of radiotherapy and the closing date, ranged between 11 and 34 (median: 19) months. In 10 patients a treatment failure was detected. Six patients had a recurrence at the primary tumor site, two patients had a lymph node recurrence and two patients had distant metastasis as verified by biopsies. Overall patients with hypoxic tumors had a poorer outcome compared to patients with non hypoxic tumors. Among the six patients with T-site recurrence, five had hypoxic tumors and one had a non-hypoxic tumor. The latter patient, who did have a borderline hypoxic value of 1.3, did not complete the radiotherapy treatment. The two patients with distant metastases and the two with lymph node recurrence both had hypoxic primary tumors. At the closing date five patients had died, all with recurrence. One patient with T-site recurrence is still alive after having received a salvage laryngectomy. The remaining thirty patients are still alive with no sign of recurrence.

Fig. 1 shows tumor treatment failure within the ranking of HV. All treatment failures, apart from one (who did not complete treatment), where found among the hypoxic tumors. The actuarial 30-month loco-regional control values were 93% and 66% ($p = 0.07$) for patients with non hypoxic and hypoxic tumors, respectively. However, the corresponding disease free survival values showed a significant difference in hypoxic and non hypoxic tumors (Fig. 4).

Discussion

A key point in hypoxia specific imaging is the evaluation method to differentiate hypoxic from non-hypoxic tumors. Typically a tumor-to-background index is used. Based upon a certain threshold the voxels within the tumor exhibiting a ratio value above the threshold define a hypoxic volume (HV). Some studies using FAZA imaging have used a qualitative measurement for FAZA uptake [20], however, this method is highly dependent on the user and the PET window settings, and hence it is not very reproducible. Other studies have used a threshold based upon thresholds used in FMISO imaging which typically range from 1.2 to 1.4. Since the authors of those studies expected higher T/M ratios in FAZA PET compared to FMISO the threshold for hypoxia was arbitrarily set to 1.5 [21,22]. Using a threshold for defining hypoxia is not biologically meaningful, since neither hypoxia nor tracer retention is a dichotomic phenomenon. However, it illustrates that some tumors contain more hypoxia than others.

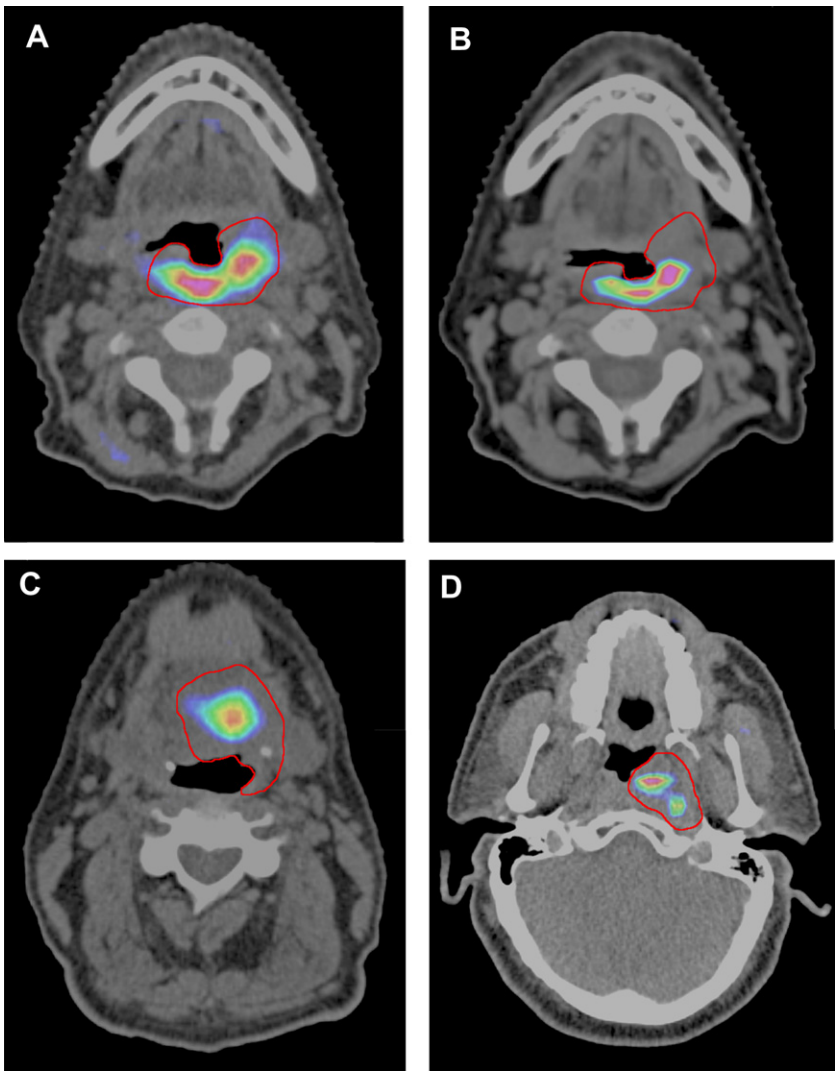

Fig. 3. Top panel: Example of repeated FAZA PET/CT scans of a patient (ID 10) with oropharyngeal cancer. The red line illustrates the GTV. (A) The hypoxic volume (T/M $\geqslant$ 1.4) is 30.9 cm^3 at baseline. (B) After 12 Gy the hypoxic volume has decreased to 13.7 cm^3, but the localization remains stable. Bottom panel: FAZA PET/CT scans of patients with tumors having a hypoxic volume (FAZA T/M $\geqslant$ 1.4). (C) Male (ID 1) with a HPV-positive oropharyngeal cancer. (D) Male with a HPV-negative oropharyngeal cancer (ID 31).

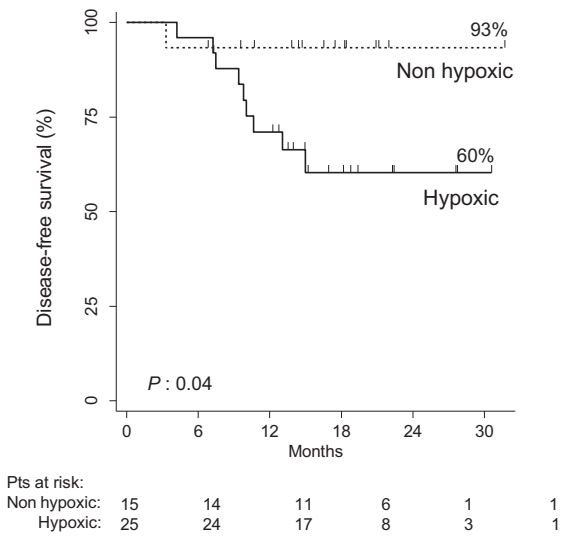

Fig. 4. Disease free survival of patients with hypoxic versus non hypoxic tumors as assessed by FAZA PET.

In this study, the threshold was selected based upon non-hypoxic normal tissue. A neck muscle was used as the reference tissue, as done in previous studies [21,22]. The threshold separating hypoxia from non-hypoxia was determined as three standard deviations of the pooled normalized muscle values for all 40 patients. More than 99% of the muscle voxels fell below 1.4. Consequently the hypoxic volume within the tumor was defined as all voxels with a T/M equal to or above 1.4. Hence all tumors containing one or more voxels with a value of 1.4 were identified as hypoxic. In a previous study investigating small tumors (50 mm^3), which resembles the size of a voxel, higher doses were required for tumor control in hypoxic compared to normoxic tumors [23]. The validity of this threshold obviously requires correct delineation of the reference volume in the neck muscle and further confirmation is warranted. Of interest is the observation that the only patient with a non hypoxic failure had a T_{max}/M value of 1.3.

We have previously showed hypoxia assessed by FAZA PET is prognostic for outcome in tumor bearing mice [24]. This is the first study to investigate the prognostic value of FAZA PET/CT imaging in patients with HNSCC and we found FAZA PET/CT to be prognostic for disease free survival in HNSCC. Significant higher T classification was found among the hypoxic tumors. But the tumor

volume based upon CT was not different between the groups; consequently in this study hypoxic status was independent of the volume of the tumor. To test the independent prognostic value of FAZA PET/CT may require a multivariate analysis which is not be possible due to the low number of events. Other clinical studies have investigated the prognostic value of hypoxia specific imaging. Generally these studies only include a small number of patients. However, despite using different tracers, different types of scannings (static/dynamic), various discriminators for identifying the hypoxic tumors and different endpoints the results of these studies are in line with our result; a tendency for a poor response to radiotherapy for tumors that were hypoxic [25].

The hypoxic radiosensitizer nimorazole was given to all 40 patients, as this is part of the standard radiotherapy treatment for HNSCC in Denmark. In a previous study by Nordsmark et al., with a similar patient cohort also receiving nimorazole, a significant difference between hypoxic and non-hypoxic tumors could be detected [18] thus our study was designed accordingly but not accounting for HPV status. HVP-positive tumors have a more favorable prognosis in terms of tumor control and survival compared to HPV-negative cancers [14], and the incidence of these tumors is rising. This was reflected in the current study as 16 of the tumors were HPV-positive. As a consequence, the treatment outcome in the DAHANCA 24 study is properly better than initially anticipated. No correlation between HPV status and hypoxia was found in the current study. Toustrup et al. showed some HPV-positive tumors does express a hypoxia specific gene profile, also indicating hypoxia can be present in these tumors as well as in the HPV-negative tumors [17]. Nevertheless, one study found HPV-positive tumors had a favorable prognosis regardless of hypoxic modification [26] presumably due to increased tumor radiosensitivity. In the current study, two patients with hypoxic HPV-positive tumors experienced treatment failure. Both had a history of smoking which is in line with a study showing a poorer outcome in patients with HPV-positive tumors and a smoking history as compared to patients with HPV-positive tumors alone [27].

Overcoming hypoxia by dose painting is an intriguing concept. Hypoxic cells are more radiation resistant; hence by increasing the dose to hypoxic volumes of the tumor a higher tumor control is expected [25]. As a result, some studies have investigated the feasibility of dose painting a tumor based upon a hypoxia specific PET scan and concluded that it is feasible [22,28], but whether it will improve outcome remains to be proven [25].

In the present study, static FAZA PET/CT scans were used to identify hypoxia. It is essential that FAZA only is retained in hypoxic cells. This has previously been illustrated in an in vitro study where FAZA uptake was found to be highly hypoxia specific and to display little cell-to-cell type variability [29]. In accordance with this, Busk et al. found a significant positive correlation between the spatial distribution of FAZA autoradiography and pimonidazole (a well established exogenous hypoxia marker) in four different tumor models [30]. Static scans 2 h after injection of FAZA were utilized in this clinical study, although dynamic scans which allow pharmacokinetic analysis arguably allow more accurate information of hypoxia [31]. However, dynamic scans and kinetic modeling are not feasible in a daily clinical setting.

In order to identify the hypoxic tumors a tumor-to-muscle threshold was used, clearly identifying some tumors as being more hypoxic than others. This threshold is entirely dependent on the stability of the muscle reference, as a small change in the muscle reference can lead to a considerable change in size of the hypoxic volume. An additional challenge for dose painting is the day-to-day stability of the hypoxia, which has been addressed previously. In one study repeated baseline FMISO imaging 3 days apart showed a mismatch between the locations of intense FMISO uptake for seven out of 13 patients [32]. However, in tumor-bearing mice a strong voxel-to-voxel correlation of FAZA PET could be found in repeated baseline scans [33]. Also, the possible change of location of hypoxia over the course of radiotherapy is essential to investigate. Koh et al. showed a general decline in FMISO FHV among patients with non small cell lung cancer in six patients, but in one patient the FHV was higher at the end of treatment compared to baseline [34]. In our study the repeated scans showed a general decline in the hypoxic volume at the second scan compared to the baseline hypoxia. Detectable hypoxia could be seen within the tumor at the second scan in six patients, and generally the location of the hypoxic volume remained stable. However, the results are hampered by the small patient number, the variability in time between scans and lack of voxel-to-voxel correlation.

Another challenge is the size of the PET voxel. A single PET voxel may represent the average value of FAZA uptake in cells ranging from anoxic to fully oxygenated with an unknown mixture of necrotic tissue. Thus, as previously shown in pre-clinical models, small regions of hypoxic cells can 'hide' within a non-hypoxic voxel [35]. Consequently, PET imaging by FAZA and similar PET tracers can describe hypoxia, but lack of uptake on a clinical PET scanner does not necessarily equal lack of hypoxia. Hence, if a tumor contains a hypoxic volume, dose boosting the entire tumor seems be the appropriate strategy to assure all hypoxic cells are targeted [25]. One modeling study concludes moderate dose escalations may improve the tumor control probability [36] but this needs clinical verification [25].

The relationship between hypoxia as detected by FAZA PET/CT imaging in a lymph node and the primary tumor was also investigated in the present study. In the majority of patients there was agreement between the detection of a hypoxic volume or lack thereof, but in nine out of 30 patients there was no correlation between the two, when using the HV as the hypoxic discriminator. This finding is in accordance with a previous PET study [37] and emphasizes the importance of investigating both the primary tumor and lymph node, since the hypoxia in a lymph node might not be representative for the oxygenation status of the primary tumor. However, a study using the Eppendorf electrode showed a good correlation between the oxygenation status of a lymph node and the primary tumor in patients [38], hence further studies investigating this issue are needed.

In this prospective clinical study FAZA PET/CT imaging was utilized to identify hypoxia in 40 patients with HNSCC receiving radiotherapy. A large inter-tumor variability in FAZA uptake could be detected and was associated with poor outcome in patients with hypoxic tumors. This study emphasizes the role of FAZA PET/CT imaging as a suitable assay with prognostic potential for detection of hypoxia in HNSCC.

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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.2012.09.015.

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