PET-based analysis of tumor glucose metabolism and tumor hypoxia before and during anti-neoplastic treatment
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Chapter 5

Dynamics of tumor hypoxia assessed by FAZA PET/CT in head and neck and lung cancer patients during chemoradiation: Possible implications for radiotherapy treatment planning strategies.


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ABSTRACT

Purpose: To define the optimal time point for the integration of hypoxia FAZA-PET/CT information into radiotherapy treatment planning to benefit from hypoxia modification or dose escalation treatment. Therefore, we performed a prospective cohort study, using serial hypoxic imaging [FAZA-PET/CT] prior to and at several time-points during (chemo) radiotherapy (CHRT) in six head and neck squamous cell (HNSCC) and six non-small cell lung cancer (NSCLC) patients.

Materials and Methods: The spatio-temporal dynamics of tumor hypoxia and fractional hypoxic volumes (FHV) were evaluated using a voxel-by-voxel analysis based on a FAZA-T/B ratio of 1.4 at four time points in HNSCC patients, at baseline (FAZA-BL), at week one (FAZA-W1), two (FAZA-W2), and four (FAZA-W4) during CHRT and at three time points in NSCLC patients (baseline; W2, W4).

Results: Ten out of twelve patients has showed substantial pre-treatment tumor hypoxia representing a FHV ≥ 1.4 assessed by FAZA-PET/CT. The median FHV was 38% (FAZA-BL), 15% (FAZA-W1), 17% (FAZA-W2) and 1.5% (FAZA-W4) in HNSCC patients, and 34% (FAZA-BL), 26% (FAZA-W2) and 26% (FAZA-W4) in NSCLC patients, respectively. Stable tumor hypoxia was observed in three HNSCC patients and two NSCLC patients at FAZA-W2. In three HNSCC patients and two NSCLC patients FHVs declined to non-detectable hypoxia levels at FAZA-W4 during CHRT, while two NSCLC patients, showed increasing FHVs.

Conclusion: Our results indicate that, instead of using the FAZA-BL scan as the basis for the dose escalation, FAZA-W2 of CHRT is most suitable and might provide a more reliable basis for the integration of FAZA-PET/CT information into radiotherapy treatment planning for hypoxia-directed dose escalation strategies.
INTRODUCTION

Hypoxia exists in approximately 60% of human tumors [1]. In vitro and in vivo studies have shown that tumor hypoxia is associated with increased resistance to ionising radiation and various types of chemotherapy with subsequently higher rates of local and distant failure [2,3]. In human tumors, two types of hypoxia can be distinguished, including acute and chronic hypoxia. Acute hypoxia develops as a result of a temporary microvasculature shutdown and local disturbances in perfusion, resulting from periodic episodes of opening and closing of blood vessels, which creates dynamic or intermittent hypoxia [2,4,5]. In contrast to acute hypoxia, chronic hypoxia results from increased diffusion distances between the blood vessels and the tumor cells. This causes low oxygen tension levels in the tumor leading to initiation of hypoxic signaling pathways and increased radioresistance [4].

Tumor oxygenation patterns can be heterogeneous. First, the amount of hypoxia present in tumors remarkably differs from patient to patient [6-10]. In addition, the intratumoral distribution of hypoxia may also depend on histologic tumor type [11]. Second, the severity of hypoxia in tumor cells depends on the increased diffusion distance between tumor and blood supply. Third, the distribution of oxygen status is variable, some parts of a tumor exhibit higher levels of hypoxia than others due to differences in dynamic blood flow as a result of periodic episodes of opening and closing of blood vessels. Finally, the tumor cells that are hypoxic today may or may not be hypoxic at subsequent time points due to a changing tumor microenvironment and/or to treatment itself.

At present, dedicated hypoxia PET tracers are available to visualize tumor hypoxia, of which the Nitroimidazoles, fluormisonidazole (FMISO) and fluoroazomycin arabinoside (FAZA) are the most frequently used [6,7,12,13]. However, the dynamics of hypoxic areas during treatment remain to be determined. One of the strategies to overcome hypoxia induced radioresistance may be dose escalation to hypoxic areas. However, given data suggesting changes in tumor hypoxia may vary during the course of treatment, it is essential to distinguish geographically stable and dynamic hypoxia during therapy, because the choice of a dose escalation strategy to hypoxic areas will strongly depend on the dynamics of tumor hypoxia during the course of radiation. Thus, hypothesizing that tumor hypoxia measured by FAZA PET/CT might guide radiation dose escalation to more radioresistant parts of tumors; this study was designed to determine spatio-temporal dynamics of tumor hypoxia during (chemo) radiotherapy (CHRT). To this end, we performed a prospective cohort study using serial hypoxic imaging before and at several time-points during CHRT in head and neck squamous cell carcinoma (HNSCC) and non-small cell lung cancer (NSCLC).
MATERIALS AND METHODS

Patients

From December 2011 to March 2013, we included 12 patients (8 male and 4 female) in this prospective observational cohort study with a median age of 58 years (range 47-68). All patients included in the study had stage IV HNSCC (n=6) or stage III or IV NSCLC (n=6) and were treated with concurrent CHRT according to our institutional protocols. The study protocol was approved by the ethical committee of the University Medical Center Groningen. All patients gave written informed consent prior to participation in the study. The patient characteristics and imaging data are shown in Table 1a (HNSCC) and 1b (NSCLC).

Scheduling of FAZA Imaging

The imaging schedule for the HNSCC and NSCLC patients is presented in figure.1 All patients underwent baseline FAZA-PET/CT before CHRT referred to as FAZA-BL. In the HNSCC patients, repeat FAZA-PET/CT scans were made during treatment at week 1 (FAZA-W1), week 2 (FAZA-W2) and week 4 (FAZA-W4) after the start of treatment. NSCLC patients underwent repeat FAZA-PET/CT scans at 2 weeks (FAZA-W2) and 4 weeks (FAZA-W4) only. All PET/CT scans were carried out on a Biograph Sensation (Siemens Medical Solutions, Hoffman Estates, Knoxville, TN, USA).

FDG-PET/CT

FDG-PET scans were made at the Department of Nuclear Medicine and Molecular imaging (NMMI) of the University Medical Center Groningen (UMCG) on a (Siemens, Germany) Biograph mCT machine

FIGURE 1: Imaging schedule for the HNSCC and NSCLC patients
and executed according to EANM guidelines [14]. Blood samples were taken before tracer injection to confirm an acceptable blood sugar level (<11 mmol/l) after an overnight fast minimum of 5 to 6 hours. Patients were injected with 3 MBq/kg bodyweight intravenously. After a waiting period of 60 min, a scan was made from the mid-thigh to the brain. The SUVmax was obtained by delineating the volume of interest comprising the entire tumor volume. The data were reconstructed with an iterative ordered subsets expectation maximization (OSEM), TOF (Time of Flight) + HD (High Definition) reconstruction algorithm with three iterations, 21 subsets with 8 mm Gaussian post-filter (NEDPASS Protocol) with a spatial resolution of 2.04 × 2.04 × 2 mm³. All PET and CT scans were analyzed using the MIM Vista software (MIM corp., Version 6.1, Ohio, USA), a computer-based workstation for visualization, quantification, and analysis of PET/CT images.

FAZA-PET/CT

FAZA-PET/CT scans were also performed on the same machine as the FDG-PET images mentioned above according to EANM guidelines [14]. Patients were injected with 370 MBq intravenously. After a waiting period of 120 min, a scan was made from the mid-thigh to the brain and analyzed using the above-mentioned research workstation. FAZA SUVmax was estimated in the same way as with FDG-scans, including correction for the partial volume effect. The median time interval between FDG-PET/CT and FAZA-BL scan was 4.5 days (range: 1-17). The data were reconstructed with an iterative ordered subsets expectation maximization (OSEM), TOF (Time of Flight) + HD (High Definition) reconstruction algorithm with three iterations, 21 subsets with 5 mm Gaussian post-filter with a spatial resolution of 2.04 × 2.04 × 2 mm³.

Assessment of voxel based spatial correlation between FAZA-PET/CT scans

To allow a voxel-wise correlation of tumor SUV values between the different FAZA PET/CT scans, the gross tumor volume (GTV) was defined on the baseline FDG PET and CT data using a threshold of 34% (NSCLC) and 40% (head and neck) [15,16] for the maximal SUV value within a VOI containing all suspected tumor tissue. To determine which voxels of each follow-up FAZA PET/CT corresponded with the voxels within the VOI determined on the baseline FDG PET/CT, FAZA PET/CT datasets were aligned with the baseline FDG data using the corresponding CT datasets in a two-step procedure based on previously published methodology [6]: First, the CT of the FAZA PET/CT was co-registered to the CT of the FDG PET-CT using a rigid registration. In a second step, CT-CT registration was adjusted using an intensity-based, free-form deformable image registration (DIR) algorithm (MIM VISTA 6.1 corp). The results are cross validated with the Mirada Medical imaging software). The resulting deformation field was then applied to the FAZA PET data. In this way, voxels of the deformed FAZA PET/CT datasets were aligned with the voxels of other FAZA-PET scans.
Inverse consistency of deformable image registration of FAZA-PET/CT for voxel based analysis

The CT data of the sequentially acquired integrated PET/CT data were used to calculate the deformation vector field between imaging time points. The deformation vector field was subsequently applied to the PET data. Apart from visual inspection of the deformation vector field and the deformed image, an inverse consistency check was done to assess the performance of the deformation algorithm [17]. The FDG-PET tracer uptake per voxel was used as a spatial marker for that specific voxel. Invertibility of the transformation was assessed by determining the forward free form deformation which maps the baseline FDG-PET/CT data back onto the FAZA-PET/CT next to the backward free form deformation mapping the FAZA-PET/CT onto the baseline FDG-PET/CT dataset. Applying the forward and backward transformations sequentially to the original FDG-PET data should restore the original FDG-PET data as close as possible. To evaluate this, Spearman's correlation was determined between the PET tracer uptake values of spatially corresponding voxels of the original and transformed FDG-PET data. Since it is not straightforward to track voxels individually, we assumed that in case of a proper free form deformation, original tracer uptake values should be restored as good as possible after forward and backward transformation and therefore the correlation coefficient between original and transformed voxel values should approximate the value of one as close as possible.

Calculation of fractional hypoxic volume

The Fractional Hypoxic Volume (FHV) was defined as the volume within the GTV exhibiting a tumor-to-background [T/B] ratio ≥ 1.4 on the FAZA scans [6,8,12,13]. The FHV of the tumor was determined along the following steps. First, the original baseline GTV on CT was created and the standardized uptake values within the GTV were expressed per voxel. Next, a tumor free area in the mediastinum of at least 30 mm diameter was chosen as a reference background in NSCLC patients and neck muscle was chosen as a reference background in HNSCC patients. The mean SUV of this background area was calculated. Finally, the FAZA T/B ratio was assessed by calculating the ratio between the SUV within the GTV and SUV_{mean background}.

Spatial-temporal dynamics of tumor hypoxia

Based on the FAZA T/B ratio cut-off ≥ 1.4, we produced FAZA-FAZA scatter plots divided into four quadrants visualizing tumor dynamics during treatment (figure 2): Quadrant A contains voxels that were non-hypoxic at baseline but became hypoxic during treatment, corresponding with increased hypoxia. Quadrant B contains voxels that were hypoxic at baseline and remained hypoxic during treatment; this was considered to represent stable hypoxia. Quadrant C contains voxels that were non-hypoxic at baseline and remained non-hypoxic during treatment regarded as stable non-hypoxia.
Finally, quadrant D contains voxels that were hypoxic at baseline but became non-hypoxic during treatment; this was considered to represent decreasing hypoxia.

**FIGURE 2.** FAZA-FAZA scatter plots divided into four quadrants visualizing tumor dynamics during treatment based on the FAZA T/B ratio cut-off $\geq 1.4$.

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**RESULTS**

Ten out of 12 patients has showed substantial pre-treatment tumor hypoxia representing a FHV $\geq 1.4$ assessed by FAZA-PET. The baseline FHVs ranged from 5% to 85%, (median 38%) in HNSCC patients, and from 0.3% to 64% (median: 34%) in NSCLC patients. The serial FHVs during CHRT are listed in Tables 1a and 1b. The median FHV at FAZA-W1, FAZA-W2 and FAZA-W4 was 15%, 17% and 1.5% in HNSCC patients, and 26% (FAZA-W2) and 26% (FAZA-W4) in NSCLC patients respectively.

In terms of invertibility of the free form deformable image registrations, correlations between original and transformed tracer uptake values of the baseline FDG PET were all significant (Spearman correlation $p < 0.001$) with an average correlation coefficient of $0.76 \pm 0.26$ (mean $\pm$ SD). No significantly different correlation coefficients were observed between head and neck PET data and lung PET data nor for the transformations determined for the PET data at different time points.
FIGURE 3. Decreasing tumor hypoxia based on FAZA-FAZA scatter plot and its corresponding transaxial FAZA-PET/CT image in patient #4 [HNSCC].

FIGURE 4. Stable hypoxia based on FAZA-FAZA scatter plot and its corresponding transaxial FAZA-PET/CT image in patient #3 [HNSCC].
Dynamics of tumor hypoxia in head and neck

All six patients underwent the FAZA-BL scan. Out of these six, four underwent the FAZA-W1, five the FAZA-W2, and four the FAZA-W4 (Table 1a). In one patient [#1], a stable non-hypoxia was observed in which the majority of voxels within the GTV were non-hypoxic (95%) at the FAZA-BL and remained such until the end of the treatment course.

In patient #2, a stable hypoxia was observed at FAZA-W1, when compared with FAZA-BL. FHV was 50% at FAZA-BL, declining to 28% on FAZA-W1, and to 5% at FAZA-W2. In this patient, eventually all voxels within the VOI became non-hypoxic at FAZA-W4. In patient #4, 85% of all voxels were hypoxic at FAZA-BL, decreasing to 24% at FAZA-W2, and 5% at W4 (decreasing hypoxia [figure 3]). In addition, in both patients [#2 and #4], a significant reduction in the FAZA T/B ratio was observed on FAZA-W2 and FAZA-W4 as compared to FAZA-BL, additionally indicating decreasing hypoxia [data not shown].

In two patients [#3 and #5], voxels which had been hypoxic at the FAZA-BL remained such at the FAZA-W2, suggesting a stable hypoxic area (figure 4). In contrast, at FAZA-W4, 98% of all voxels within the VOI became non-hypoxic in patient #5, suggesting a stable non-hypoxic pattern. The remaining patient [#6], who had only two FAZA-PET/CT scans available (FAZA-BL and FAZA-W1), also showed a stable non-hypoxia with a FHV of 18% at FAZA-BL, even decreasing to 1% at FAZA-W1.

Dynamics of tumor hypoxia in NSCLC

All six patients completed FAZA-BL and FAZA-W2, four patients also underwent FAZA-W4 (Table 1b). Two patients (patient’s #1 and #2) showed no hypoxia at FAZA-W2 when compared with FAZA-BL (figure 5). Patient #1, started with an FHV of 33%, but this decreased to 0% at FAZA-W2 and remained such during the treatment course, thus exhibiting a stable non-hypoxia.

In patient #3, dynamic hypoxia was observed. The FHV of the tumor decreased from 63% at FAZA-BL to 26% at FAZA-W2, and again increased to 46% at FAZA-W4. This might indicate a highly variable tumor microenvironment, in some tumors. In patient #4, 31% of the voxels which were hypoxic at FAZA-BL remained so at FAZA-W2, thus exhibiting stable hypoxia.

In patient #5, dynamic hypoxia was observed, with 42% of the non-hypoxic voxels at FAZA-BL becoming hypoxic at the FAZA-W2. The two above-mentioned patients [#4 and #5] underwent only two FAZA-PET/CT scans (FAZA-BL and FAZA-W2), making any further interpretation impossible.

In the remaining patient #6, 6% of the FAZA-BL non-hypoxic voxels became hypoxic at FAZA-W2.
FIGURE 5. Stable non-hypoxia based on FAZA-FAZA scatter plot and its corresponding transaxial FAZA-PET/CT image in patient #2 (NSCLC).

FIGURE 6. Increasing tumor hypoxia based on FAZA-FAZA scatter plot and its corresponding transaxial FAZA-PET/CT image in patient #6 (NSCLC).
### TABLE 1A. Patient Characteristics and Imaging Data of HNSCC patients

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<th>Patient no</th>
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<th>Pretreatment Lesion size (cm)</th>
<th>Pretreatment Stage</th>
<th>Baseline FAZA T/B</th>
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<th>FHV</th>
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Abbreviations: FDG = Fluorodeoxyglucose; SUVmax = Maximum standardized uptake value; FAZA = fluoroazomycin arabinoside; T/B = Tumor to Background ratio; FHV = Fractional hypoxic volume; *The patient received no induction chemotherapy.

### TABLE 1B. Patient Characteristics and Imaging Data of NSCLC patients

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Abbreviations: FDG = Fluorodeoxyglucose; SUVmax = Maximum standardized uptake value; FAZA = fluoroazomycin arabinoside; T/B = Tumor to Background ratio; FHV = Fractional hypoxic volume; *The patient received no induction chemotherapy. **Stereotactically sterilized solitary brain metastasis.
Subsequently, 35% of the non-hypoxic voxels at FAZA-W2 became hypoxic at FAZA-W4, indicating increasing tumor hypoxia (figure 6).

In summary, there was a general trend toward decreasing tumor hypoxia in HNSCC patients during treatment, with a majority of non-hypoxic voxels within the VOI and remained so for the duration of the treatment. However, in NSCLC patients, two patients showed already quite low FHV's at baseline, which may or may not be due to the two courses of induction chemotherapy. FHV's in these tumors declined to non-detectable hypoxia levels at FAZA-W2 during chemoradiation. Two other patients, showed no apparent decrease in serial FHV.

DISCUSSION

To our knowledge, the current study is the first to report on the spatio-temporal dynamics of tumor hypoxia as detected by FAZA-PET/CT and was monitored over a prolonged period of observation in six head and neck and six NSCLC patients during the course of primary CHRT.

In the present study, a detailed voxel-by-voxel analysis of FAZA-PET/CT scatter plots manifested intratumoral hypoxic distribution. In short, four different types of cases were found: (1) increasing hypoxia; (2) decreasing hypoxia; (3) stable hypoxia; and (4) stable non-hypoxia. Recently, Bittner et al. [10] prospectively studied the capacity of FMISO-PET/CT to determine the changes in hypoxic subvolumes in head and neck cancer patients during the course of treatment. Three out of five patients showed both a stable and dynamic hypoxia on the second FMISO-PET/CT scan and a stable hypoxia was observed in remaining two patients. Similar results were reported by Zips et al. [9], using FMISO-PET/CT underlying the heterogeneous behavior of tumor hypoxia.

The question arises what the optimal time point will be for hypoxia imaging during treatment, to obtain optimal integration of repeat FAZA-PET/CT information into radiotherapy treatment planning for the purpose of dose escalation strategies. In our study, ten out of twelve patients showed substantial tumor hypoxia at FAZA-BL within the GTV. Dose escalation to the whole GTV will be less attractive as this will result in higher radiation doses to the normal tissues as well and subsequently to an increase in radiation-induced side effects. On the other hand, tumor hypoxia may vary during the course of radiotherapy, which, from a theoretical point of view would make dose escalation more effective. In this study three out of six in HNSCC patients and four out of six in NSCLC patients showed detectable hypoxia in the FAZA-W2 (week 2) of CHRT. In this regard, a well demarcated hypoxic area at FAZA-W2 of CHRT seems to be more appropriate and can be treated with spatially conformed doses by precisely targeting tumor hypoxia. A similar time point has been proposed by Bittner et al. [10] and Zips et al.
Dynamics of tumor hypoxia in HNSCC and NSCLC

They suggested that anticipating dynamics of tumor hypoxia on the basis of baseline FMISO-PET/CT would be inappropriate; because the majority of patients exhibit substantial tumor hypoxia at baseline and it remains questionable which parts of the tumor remain hypoxic. Although we tried to amend scheduled FAZA-PET/CT scans as per protocol, this was not always feasible in all patients because of patient logistics and the fact that the accrual rate was very low and that the same accounts for the compliance in a highly selected cohort of very motivated patients.

In our study, most tumors voxels that were hypoxic at the time of irradiation showed improved oxygenation status two weeks after the start of treatment and that remained unchanged over the subsequent duration of treatment. In addition, it was evident that CHRT resulted in a significant decrease in the turnover rate of voxels from hypoxic to non-hypoxic. Therefore, in many tumors, a treatment-induced improvement from hypoxic to non-hypoxic tumor status will affect the efficacy of the subsequent dose delivered to tumor. It has been reported that the oxygenation status of the tumor dramatically improved after radiotherapy as a result of the death of well oxygenated tumor cells [18,19].

Locoregional treatment is the mainstay of cancer treatment in non-small cell lung cancer and head and neck cancer. For locally advanced disease, dose intense multimodality treatment is required, although it is affected by substantial side effects. The goal is to shift away from the current paradigm of delivering a homogeneous dose distribution to the tumor toward the delivery of more spatially conformed radiation doses to radioresistant areas of tumor [20]. This will thus allow the delivery of much higher doses to tumors without increasing the adverse side effects on adjacent normal tissues. Such an approach may improve the locoregional tumor control of advanced stage tumors providing biological rationale for dose escalation strategies [21]. However, it has also been postulated that presence of tumor hypoxia generally reflects a more radioresistant tumor phenotype requiring dose escalation of the entire tumor volume rather than boosting the hypoxic areas only [22]. Unfortunately, these hypothesis have not been proven yet. To our knowledge, no studies have been performed on how hypoxia imaging could be used to intensify the radiotherapy to radioresistant hypoxic subvolumes. Therefore, future studies should focus on the dynamics of tumor hypoxia in order to select the most optimal treatment technique.

Further technological advances in radiation oncology have led to future visions, that are now starting to be realized and that could improve tumor control by using information on hypoxia. For instance, incorporation of more conformal radiation delivery techniques, such as intensity modulated radiotherapy (IMRT), conformal arc techniques such as VMAT and Rapid Arc, tomotherapy and proton therapy into clinical practice has enabled radiation oncologist to deliver more spatially conformed
radiation doses often referred as "dose painting" to hypoxic areas of tumor [23]. To our knowledge, no dose escalation studies have been performed on specifically targeting FMISO-PET/CT or FAZA-PET/CT identified hypoxic subvolumes in HNSCC or NSCLC. Another example is the application of serial hypoxic imaging, which might be of considerable importance in accurately stratifying patients into high and low risk groups, and therefore assist in selecting treatment regimens that could produce optimal outcomes of improved response and minimizing morbidity from futile and expensive therapies.

A voxel-by-voxel analysis is only a valid approach if corresponding tumor voxels are considered for comparison. Therefore different patient positioning between scans, tumor displacement and treatment induced changes of tumor volume need to be accounted for. One could consider imaging procedures where patient positioning during scanning is replicated using laser guides. However, these imaging procedures require additional staff training and more elaborate patient preparation. Another option is to completely rely on software to map corresponding tumor voxels. Our approach is a two step approach where first a rigid registration is applied to the whole body CT scan such that differences in patient positioning between the two PET scans are accounted for. Once translation and rotation parameters are optimized to compensate differences in patient positioning, a deformable image registration is applied to the CT data to account for tumor movement and tumor volume changes.

To allow a voxel-by-voxel analysis, tumor volume changes need to be limited such that deformable registration will only compensate for local tumor movement [24,25]. Compensation for tumor volume changes should be avoided since it is uncertain how the deformable registration deals with the discrepancy in the number of tumor voxels. To check whether functional information within the tumor volume is not affected by the deformable registration we performed a straightforward inverse consistency check by applying a deformable registration in the reverse direction and look at the correlation between the original and voxel values transformed by a forward and backward deformable registration. For this study, correlation coefficients were significant and acceptable with Spearmen's correlation coefficient = 0.76 ± 0.26 (mean ± SD), suggesting that the functional information within the tumor volume is preserved. However larger tumor volume changes due to successful therapy could pose limitations to this approach.

CONCLUSION

In conclusion, we observed relatively stable tumor hypoxia at FAZA-W2 of CHRT when compared with FAZA-BL in three HNSCC patients and two NSCLC patients. Hence, instead of using the FAZA-BL scan as the basis for the dose escalation, FAZA-W2 of CHRT is most suitable and might provide a more reliable basis for the integration of FAZA-PET/CT information into radiotherapy treatment planning for hypoxia-
directed dose escalation strategies. However, additional studies are needed on the spatio-temporal dynamics of tumor hypoxia, to stratify patients who may benefit from hypoxia based radiotherapy treatment planning before implementation.

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