

Imaging of tumor hypoxia with PET

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CHAPTER 10

General discussion and summary

GENERAL DISCUSSION AND SUMMARY

Tumor cell hypoxia is known to be a major factor that negatively influences treatment effectiveness, it promotes resistance to surgery, radiotherapy and chemotherapy and increases tumor aggressiveness, angiogenesis, and metastatic potential, resulting in a poor prognosis (1, 2). Tumor hypoxia is present in the majority of solid lesions. Detection and quantification of tumor hypoxia could help selecting patients who may benefit from hypoxia targeting treatment (3, 4). Non-invasive positron emission tomography (PET) imaging techniques provide the opportunity to perform repeated tumor hypoxia measurements, additionally it allows visualization of the spatial distribution of hypoxia (5, 6).

Several 2-nitroimidazoles, labeled with fluor-18 [^{18}F], have already been applied to identify hypoxia (5, 7). The selective binding and retention of 2-nitroimidazoles allows detection and quantification of tumor hypoxia prior to and during treatment (8-10). An extensive literature overview of hypoxia PET imaging, using 2-nitroimidazoles is given in chapter 2. The preclinical and clinical use of hypoxia PET imaging is described, with a focus on its validation, quantification and (clinical) applications. From this study we observed that several 2-nitroimidazole-based PET tracers have the ability to reliably measure tumor hypoxia, that these tracers have a prognostic value for treatment outcome in several cancers and that it is feasible to use hypoxia PET imaging to select patients for additional anti-hypoxia treatment.

Comparison of hypoxia PET tracers

The diversity in the available hypoxia PET tracers raises the question which tracer is the most optimal. In the available literature a large variety of different tracers, tumor models or patient populations were used, in addition, the scan time points and image analysis varied (5, 11). Therefore, there is a need for comparative studies, investigating different hypoxia PET tracers within one tumor model using the same acquisition protocol and analysis parameters. We performed an extensive comparison of the frequently used hypoxia PET tracers [^{18}F]HX4, [^{18}F]FAZA and [^{18}F]FMISO within one preclinical (rat rhabdomyosarcoma) tumor model. This tumor model was previously characterized and used for hypoxia PET imaging with [^{18}F]FMISO, [^{18}F]EF3 and [^{18}F]HX4 and clinical relevant fractions of hypoxia were reported (12-14). The direct comparison of the tracers, described in chapter 3, provides more insight into the strengths and weaknesses of the hypoxia PET tracers. We observed that [^{18}F]HX4 provides a significantly higher tumor-to-background ratio in comparison to [^{18}F]FAZA or [^{18}F]FMISO at the same image time point. These results are in agreement with the clinical observation of Chen et al. which showed that [^{18}F]HX4 at 1.5h p.i. reached the same contrast than [^{18}F]FMISO at 2h p.i. (15). Carlin et al. (16), on the other hand, reported no statistical difference in the tumor-to-muscle ratios of the hypoxia PET tracers [^{18}F]HX4, [^{18}F]FAZA and [^{18}F]FMISO in mice bearing SQ20b xenograft tumors. This difference might be explained by the low number of tumors (3-5 per tracer) investigated by Carlin et al. and the short injection-acquisition time interval. In chapter 3 we also observed a high repeatability of the [^{18}F]HX4 and [^{18}F]FMISO uptake in a test-retest (48h interval) setting, while for [^{18}F]FAZA lower spatial reproducibility was observed. However, in mice bearing a human SiHa cervix tumor xenograft [^{18}F]FAZA PET

imaging showed a high spatial reproducibility (17). From the current literature and our analysis in chapter 3 we can conclude that each tracer has its own strengths. Nevertheless, in clinical practice a high image contrast within a short time interval is preferred, since the patient has to wait between injection of the radioactive tracer and the scan time p.i. Also, a good (spatial) reproducibility is necessary to allow additional anti-hypoxia treatment based on hypoxia PET imaging.

Repeatability [^{18}F]HX4 PET

The ultimate aim of anti-cancer therapy is to improve the outcome of patients. We believe that targeting of tumor hypoxia with the aid of hypoxic cell sensitizers or a higher radiotherapy dose to the hypoxic regions could contribute to reach this goal. To select patients for additional anti-hypoxia therapy and to monitor the response to treatment, it is important to gain insight in the clinical day to day variability of the hypoxia PET uptake and the spatial location of the high uptake volumes. To answer this research question we performed image analysis on repeated [^{18}F]HX4 PET data, acquired within a time interval of 1 to 6 days, from a multi-centered study (NCT01075399) in patients with head and neck and lung cancer (Chapter 4). The results of this study showed a high repeatability of the [^{18}F]HX4 PET uptake parameters (SUV_{max} , SUV_{mean} , TBR, hypoxic volume), with for example a repeatability percentage of 17% and 15% for SUV_{max} and SUV_{mean} , respectively. These percentages are much lower than the repeatability expected based on [^{18}F]FDG PET images, which would be approximately 35% in the SUV range (18). A previous study with [^{18}F]FMISO in eleven patients with head and neck cancer reported similar results, showing a high reproducibility of the [^{18}F]FMISO PET parameters SUV_{max} , TBR and hypoxic volume (19). The observed repeatability of the [^{18}F]HX4 PET parameters provides evidence for a reliable detection and quantification of tumor hypoxia. [^{18}F]HX4 PET parameters on a global tumor level might therefore be able to monitor the response to treatment or guide the identification of patients who might benefit from additional anti-hypoxia treatment (4). However, for radiotherapy dose escalation also the repeatability of the PET uptake on a sub-volume or voxel level is important, since this will provide information on the spatial distribution of tumor hypoxia and the possibility to target these radio-resistant sub-volumes with a higher radiation dose (5, 20). In the patient population studied in chapter 4, we observed also a high spatial repeatability in the majority of patients. These results confirmed the observation in the preclinical study (chapter 3). However, previous repeatability studies using the alternative hypoxia tracers [^{18}F]FMISO or [^{18}F]FAZA reported contradictory results regarding the spatial uptake pattern of the hypoxia PET tracers (10, 17, 19, 21). Nevertheless, our current results show that [^{18}F]HX4 PET can reliably identify the hypoxic volumes before the start of treatment, which might benefit from treatment with an increased radiation dose.

Optimal imaging

The beneficial properties of [^{18}F]HX4, observed in the preclinical tumor model (chapter 3), the promising results of the phase I clinical trial (22) and the observed clinical repeatability (chapter 4) provide a good basis for phase II clinical trials. [^{18}F]HX4 PET imaging was incorporated as a translational research part in two clinical studies at Maastricht Clinic

(NCT01024829 & NCT01210378) and additionally a new imaging study with [^{18}F]HX4 in patients with HNSCC was initiated (NCT01347281). As mentioned previously, the available hypoxia PET data in the literature is diverse and acquisition protocols frequently vary, which makes it hard to compare and combine the published hypoxia PET studies (5, 11). Our aim was to standardize acquisition and image analysis in an early stage. Therefore we defined in the first 15 non-small cell lung cancer (NSCLC) patients the optimal image parameters for [^{18}F]HX4 PET/CT imaging (chapter 5). We observed heterogeneous but stable uptake patterns between PET images acquired at 2h or 4h p.i., indicating a high short-time reproducibility of the PET uptake. However, at 4h p.i. imaging contrast (tumor-background ratio) was superior to 2h p.i. These results were confirmed in the first 10 patients with a HNSCC, where the image contrast increased significantly up to 4h p.i. (chapter 8). In addition, preliminary results of another research group, investigating the use of [^{18}F]HX4 PET in patients with pancreatic and esophageal cancer report similar observations (AMC Amsterdam, NCT01995084, (23)).

Unfortunately, it was clinically not feasible to perform imaging beyond 4h p.i. and no information was acquired regarding the clinical [^{18}F]HX4 PET image contrast at later time points. In preclinical setting the contrast in the images remained stable from 4h to 6h p.i. (12). However, comparing the preclinical and clinical results in this thesis, we observed that the estimated half life of [^{18}F]HX4 (clearance from blood) was 2.2 hours for the rat rhabdomyosarcoma study (chapter 3), while this was 4.3 hours for the first NSCLC patients described in chapter 5. Therefore, a later acquisition time point might be beneficial in patients. Nevertheless, the continuing decay of the radionuclide would cause a decrease of the signal to noise ratio, requiring the injection of a higher tracer dose or a longer acquisition time. These technical aspects in combination with the practical disadvantages supports the decision not to increase the time interval between the [^{18}F]HX4 injection and PET scan in clinical setting.

[^{18}F]HX4 versus [^{18}F]FDG PET

Hypoxia PET imaging is still in the research stage, and is not used in routine clinical practice. [^{18}F]FDG PET, on the other hand, is a frequently used tracer to visualize tumor metabolism and may indirectly reflect the tumor microenvironment, including areas of hypoxia (1, 24, 25). We defined the hypoxic and metabolic status of NSCLC (chapter 6) and HNSCC (chapter 7), with the aid of [^{18}F]HX4 and [^{18}F]FDG PET imaging. In both studies we observed a correlation between the volume of the lesion and the overall [^{18}F]HX4 and [^{18}F]FDG PET uptake. This was in agreement with previous studies investigating the correlation between [^{18}F]FMISO PET and [^{18}F]FDG PET parameters in patients with head and neck cancer (26-28). However, for patients with NSCLC contradictory results were reported in literature (29-31). General correlations on a tumor level, however, provide no information about the spatial orientation of a high metabolism or hypoxia. Therefore additional analysis on a sub-volume level were performed evaluating the agreement between volumes with a high metabolism (uptake > 50% SUV_{max}) and tumor hypoxia ($\text{TBR} > 1.4$). Note that the thresholds to define a high metabolism and hypoxia were defined arbitrarily, based on ongoing [^{18}F]FDG boost trials (32, 33), and previous publications on hypoxia PET imaging (12, 13, 34, 35). A change in the definition will result in different high uptake volumes and results. In radiotherapy dose escalation, pre-defined thresholds are only

relevant if a 'dose painting by contour' approach is selected, treating the high uptake volumes with a higher dose level. For 'dose painting by numbers', on the other hand, thresholds do not play a role, since the dose is based on the local voxel intensity values within the tumor (36).

Nevertheless, based on our predefined threshold to define high metabolism and hypoxia a (partial) mismatch was observed in approximately 50% and 70% of the primary NSCLC and HNSCC lesions, respectively (chapter 6 and 7). The fraction of the hypoxic volume, located outside the high [^{18}F]FDG volume, was on average similar for NSCLC (24%) and HNSCC (25%). However, only in the NSCLC patients a distinct uptake pattern between the high [^{18}F]HX4 and [^{18}F]FDG uptake volumes was observed in 2 cases. This can be explained by the patient populations used in the studies. The NSCLC population consists of tumors with a heterogeneous pathology, including mainly patients with an adenocarcinoma of the lung, while the head and neck cancer patients all suffered from a squamous cell carcinoma. In the literature it is described that adeno and squamous cell carcinomas show differences in their metabolism. Where adenocarcinomas rely mainly on aerobic glycolysis, squamous cell carcinomas have a more physiologically metabolism, using mitochondrial oxidation with anaerobic glycolysis under hypoxia (37). Although we observed in both patient populations a partial agreement between the extend and location of hypoxia and metabolism, they represent different properties of the tumor and provide complementary information which can be used as a prognostic marker for outcome (2, 9, 38-40), to select patients for additional therapy (4, 41, 42) or to target the resistant (high metabolic or hypoxic) volumes within the tumor (32, 33, 43, 44).

Monitoring response

The ability to monitor the response to treatment could provide valuable information to adapt the treatment in an early stage. The results of previous studies showed that hypoxia PET imaging with [^{18}F]FMISO and metabolic [^{18}F]FDG PET imaging were able to observe changes in the uptake during (chemo)radiotherapy, and that these changes had a higher predictive value for the treatment outcome than pre-treatment measurements (9, 39, 45, 46). We performed a study to investigate the treatment-associated changes in tumor hypoxia using [^{18}F]HX4 PET imaging and hypoxia-related blood biomarkers (chapter 8). In agreement with previous literature, a significant decrease of the hypoxia [^{18}F]HX4 PET uptake was observed during treatment with radiotherapy alone or in combination with cisplatin or cetuximab. The evaluated blood biomarkers, on the other hand, were not specific enough to measure a significant decrease in tumor hypoxia during treatment. This might be caused by the included patient cohort, since the observed plasma levels of plasma osteopontin and CAIX were relative low in comparison to previous studies, reporting the osteopontin and CAIX levels in large numbers of head and neck cancer and rectal cancer patients (42, 47). In addition, plasma osteopontin is also known to play a role in the immune regulation and stress response which might also be induced by radiation (48). In our patient cohort, and based on our definition of tumor hypoxia ($\text{TBR} > 1.4$), only 2 lesions had a remaining hypoxic volume ($> 1\text{cm}^3$) during treatment. For these lesions, the localization of the hypoxic volume was (almost) completely within the hypoxic volume defined at baseline, which suggests that the localisation of persistent hypoxia is stable during treatment. This would allow the boosting of hypoxic subvolumes defined at the start of treat-

ment. However, previous studies using alternative hypoxia PET tracers showed contradictory results, reporting a stable localisation (10) or a spatial move during treatment (21, 49, 50). All studies show results of a relative low number of patients, and should be interpreted with care. Nevertheless, potential changes in the spatial distribution of hypoxia should be taken into consideration when applying hypoxia-guided dose escalation (49-51).

Targeting of tumor hypoxia

Tumor hypoxia can be targeted with the aid of hypoxia-activated prodrugs, which are activated in an environment with a low oxygen concentration. TH302 is a one of these drugs, which is activated on the reduction of its 2-nitroimidazole component, eventually releasing the active drug bromo-isophosphoramidate mustard that acts as a DNA cross-linker which leads to cell death. Several clinical Phase I and II studies have been performed, investigating the safety and the potential of TH302 in combination with chemotherapy, with promising results (52-55). Since TH302 specifically targets the hypoxic cells, which are more resistant for radiation, the combination of radiotherapy with TH302 might have also the potential to improve tumor control. In chapter 9 we studied in a preclinical setting, for the first time, the effect of radiotherapy in combination with the anti-hypoxia drug TH302. [^{18}F]HX4 PET was used to evaluate the response to treatment. In addition the relationship between hypoxia PET imaging and the therapeutic outcome was assessed. We observed that the efficiency of the treatment was dependent on the oxygenation status, where a decreased oxygenation status increased the therapeutic effect of TH302. The pre-treatment hypoxic fraction was associated with tumor growth delay (time to reach 4x startvolume) and [^{18}F]HX4 PET imaging was able to detect a decrease in the hypoxic fraction after treatment with TH302. These results confirm that hypoxia PET imaging could be used to select patients with a hypoxic tumor, which will benefit the most from the additional treatment with TH302. In addition, serial imaging of tumor hypoxia could be useful to assess the effect of TH302. These promising results will be translated to a clinical trial in patients with esophageal cancer, with the aim to assess the maximum tolerated dose and anti-tumor activity of TH302 in combination with chemoradiation. In addition the prognostic value of [^{18}F]HX4 PET imaging at baseline and after administration of TH302 will be explored.

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