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Hypoxia and tumor metabolism in radiation oncology: targets visualized by positron emission tomography

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Due to the amazing leap of technology in radiation oncology in the past few years, cancer treatment will become more individualized. Molecular imaging with PET contributed to this with its many tracers available, each of them visualizing a specific feature of a tumor and its microenvironment revealing the biological characteristics of cancer. Hypoxia is of interest as hypoxic tumor cells are associated with lower disease control because of an increased resistance to cytotoxic treatment. This is especially the case for radiotherapy. Treatment adaptations overcoming the negative effect of hypoxia have shown promising results. Several hypoxia tracers are available of which [18F]FMISO is studied most extensively, however other tracers are studied as well and the search for highly specific and reproducible PET tracers is still ongoing. Wide experience has been gained with the use of [18F]FDG PET as it is used on a routine basis for diagnosing and staging of cancer. Although not a specific marker for hypoxia, increased metabolic rate reflects increased proliferation and glycolysis indicating increased treatment resistance. Molecular imaging by means of PET creates an opportunity to provide personalized care, with optimal disease control, minimal toxicity and best cost-effectiveness.

KEY WORDS: Neoplasms - Cell hypoxia - Positron-emission tomography - Radiotherapy.

The challenge in radiation oncology is to achieve optimal locoregional tumor control with minimal toxicity. The treatment of patients with cancer is often multimodal as surgery, systemic therapy and radiotherapy are frequently combined to accomplish the best chances for cure.

For the optimization of local tumor control, radiotherapy can be applied in a (neo-)adjuvant setting to surgery. However, irradiation is also widely used in for example head and neck cancer patients providing organ preservation. In the past decades, technical improvements made it possible to treat patients more conformal; the irradiation dose can be delivered more confined to the tumor. This reduced toxicity as sparing of normal tissue improved substantially. Also, the higher conformality allowed dose escalation without increasing treatment related toxicity.

Failure to obtain local control after irradiation is due to several characteristics in the microenvironment of tumors and the tumor cells itself. Hypoxic cells are more resistant to radiation compared to well oxygenated cells.[1] In addition, enhanced repopulation is one of the features of malignant cells that compromises tumor control. The third reason for failure is the intrinsic radioresistance of tumor cells, which is related to DNA repair mechanisms. In clinical practice several approaches have been used to adapt treatment and overcome these negative features of tumor cells.

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To overcome (intrinsic) radioresistance, dose escalation by means of hyperfractionated radiotherapy schedules [2, 3] and the addition of chemotherapy [4] or a radiosensitizer have been used. Accelerated radiotherapy schedules have shown benefit as it thwarts accelerated tumor cell repopulation.[2] For counteracting the adverse effect of hypoxia, efforts have been made to improve the oxygen status of the tumor microenvironment or specifically target hypoxic cells.[5-7] However, all these adaptations have in common that only a minority of patients benefit whereas all patients experience an increase in treatment related toxicity. Therefore, it is necessary to improve patient selection for these intensified treatment approaches.

With improved imaging techniques, *e.g.* magnetic resonance imaging (MRI), it is possible to give a more precise representation of tumor spread. Except for functional MRI,[8] these anatomical imaging modalities will not represent information on the functional status of a tumor. Complementary to anatomical imaging is positron emission tomography (PET) as this imaging modality is able to give functional information of the microenvironment of a tumor on the molecular level.[9] Several tracers are available, all visualizing different aspects of the functional status of tumor cells, for example tracers for hypoxia, proliferation and metabolism[10, 11]. Combinations of these imaging modalities are potentially applicable for cancer staging, treatment selection and response monitoring. Especially treatment selection (*e.g.* hypoxia modification), radiotherapy planning (*e.g.* dose escalation) and response monitoring (*e.g.* boosting of tumor areas with remaining viable tumor cells) make it possible to individualize treatment. In this review we will discuss the use of PET imaging in radiation oncology, with the focus on tumor cell metabolism and hypoxia.

Radioresistance and PET imaging

Upfront selection of patients eligible for intensified treatment is important in preventing an increase in side effects for patients that will not gain from treatment intensification. As potential tools for treatment selection [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) PET and [^{18}F]fluoromisonidazole ([^{18}F]FMISO) PET (Figure 1) plus other hypoxia tracers will be discussed.

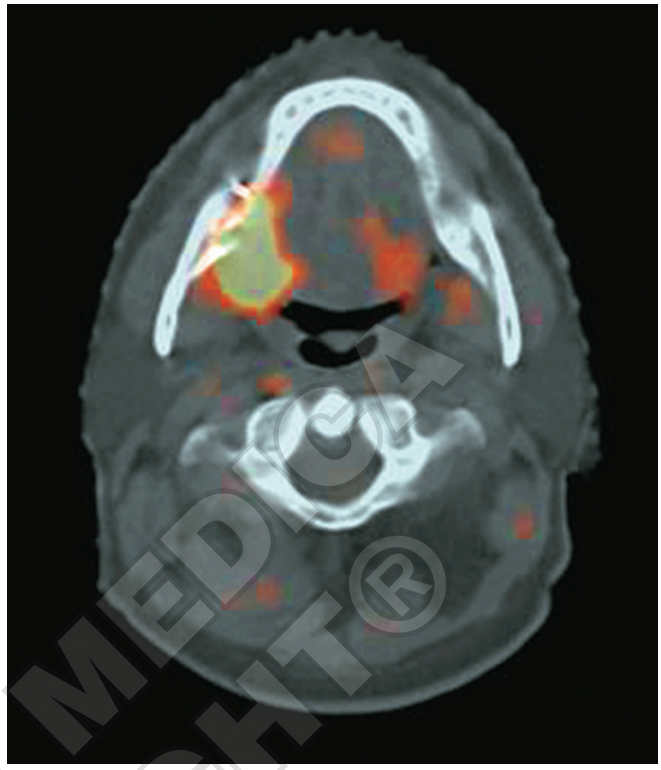

Figure 1.—[^{18}F]FMISO PET image (3 hours post injection) displaying a hypoxic carcinoma of the oral cavity in a 68 year old male.

Hypoxia and the microenvironment

Since the early fifties it is known that hypoxia limits radiation induced cell death.[1] The enhancing effect of oxygen on damage due to ionizing radiation is known as the oxygen-fixation hypothesis; it is the presence of oxygen that anchors radiation inflicted damage to the DNA.[12]

Hypoxia is heterogeneously distributed within a tumor and can change over time.[13] The blood supply of a malignant tumor is often suboptimal as the vascular network is immature and chaotic. This chaotic vascular network results in tumor hypoxia, which can arbitrarily be divided in two categories: diffusion limited and perfusion limited hypoxia. The first, also known as chronic hypoxia, is the result of proliferating cells exceeding the oxygen capacity of the newly formed vascular network. This is due to the fact that the new microvasculature is often insufficient in providing normoxic circumstances in the distant tumor areas and will thereby contribute to diffusion limited hypoxia.[14] Perfusion limited, or

acute hypoxia, is the result of structural and functional abnormalities in the newly formed vasculature causing malfunctioning of the blood supply. This in turn results in an unstable blood flow causing intermittent hypoxia close to poorly organized vessels. This form of hypoxia is characterized by rapidly changing oxygen concentrations.[15, 16]

Hypoxia can be quantified in several ways, for example direct measurement by means of oxygen electrodes,[17] or by immunohistochemical staining with hypoxic markers such as pimonidazole.[18] These two methods are invasive and are only applicable in accessible tumor areas. Also, they are not suited for longitudinal monitoring as these measurements represent smaller subvolumes of a tumor. In contrast, PET imaging is non invasive and is suitable for repetitive visualization of the complete tumor which makes it possible to give a three dimensional representation of tumor oxygenation. A disadvantage of hypoxia PET imaging lays in the fact that the tracers diffuse relatively slow and show low tumor to back ground contrast which hampers interpretation. In addition, PET has a low spatial resolution and thus does not provide information at the microregional level. The potential of non invasive hypoxia assessment by PET imaging has clear clinical advantages albeit with the abovementioned limitations.

Hypoxia and treatment outcome

Hypoxia adversely affects treatment outcome, as is shown for several solid tumors including head and neck tumors,[19, 20] cancer of the uterine cervix [21] and soft tissue sarcomas.[22] In a meta-analysis concerning 32 randomized trials with a total of 4805 patients it was shown that for head and neck carcinomas the modification of tumor hypoxia (hyperbaric oxygen, carbogen (95% oxygen and 5% carbondioxide) breathing or a hypoxic radiosensitizer) improves tumor control with an odds ratio (OR) of 0.71 (95% CI, 0.63 to 0.80; P<0.001) for locoregional control.[23] This indicates the beneficial effect of hypoxia modification, however the magnitude of the benefit differs between and within tumor sites.

Several hypoxia detection approaches and their prognostic value have been studied. Pimonidazole is an exogenous hypoxic marker and its immunohistochemical staining has proven to be of prognostic value in head and neck cancer patients. In a phase II study 2 year control rates for tumors with high hypoxic fractions, based on pimonidazole staining were 48% *versus* 87% for tumors with low hypoxic fractions.[24] The predictive value of pimonidazole immunohistochemical staining in 79 patients was shown by Janssens *et al.* in a side study of the phase III randomized Accelerated Radiotherapy with Carbogen and Nicotinamide (ARCON) trial. ARCON significantly improved regional control in patients with a high hypoxic fraction compared to patients with a high hypoxic fraction undergoing the standard accelerated radiotherapy (AR) regimen.[25]

Hypoxia induces an upregulation of proteins, which are considered hypoxia related markers. These endogenous markers, such as hypoxia inducible factor (HIF) and carbonic anhydrase IX (CA IX), can be measured in tumor tissue.[26] The exact place in hypoxia detection is subject of research. Toustrup *et al.* evaluated a 15 genes expression profile and found this profile to be capable of predicting the more hypoxic tumors to benefit most from hypoxic sensitization.[27] Plasma osteopontin concentration is considered a surrogate marker for hypoxia that can easily be measured. An inverse correlation was found as the tumor pO2 (microelectrode) was significantly lower with higher osteopontin levels in head and neck tumors. However, the correlation coefficient was 0.42 indicating that several other biochemical mechanisms might influence osteopontin concentration as well.[28] As a side study to the Danish Head and Neck Cancer (DAHANCA) 5 trial, Overgaard *et al.* found better locoregional control and disease specific survival in patients with high osteopontin levels allocated to radiotherapy in combination with nimorazole as a hypoxic sensitizer. Nimorazole did not improve outcome in patients with lower or intermediate osteopontin levels.[29] Although these results seem promising, recent published data from a side study of the TROG 02.02 randomized controlled trial could not confirm this outcome.[30] Further research is needed to clarify the role in clinical practice of the aforementioned hypoxia assessment tools.

Hypoxia and PET imaging

There are several radiolabeled tracers that can be used for hypoxia PET imaging. Used most are derivates of 2-nitroimidazole such as [^{18}F]FMISO.[31, 32] This molecule is less than 5% protein bound and free diffusible which enables a good distribution in the human body. These characteristics are of great importance for all tracers as tumor blood flow might be inadequate and might hamper distribution of

these molecules. As the tracer enters the cell it has affinity for electrons, but an even higher affinity for oxygen. In case an electron binds to the 2-nitroimidazole molecule a radical anion is formed. This action is reversed in the presence of oxygen. However, in the absence of oxygen, the 2-nitroimidazole molecule undergoes further reduction. This produces more reactive products, which are trapped intracellularly by binding to cell structures. These bound bioreduced radiolabeled tracers can be visualized with PET imaging and thus give information upon the hypoxic state of the tumor.[32] In a rat tumor model Dubois *et al.* visualized hypoxic subvolumes by means of [18F]FMISO PET. These hypoxic subvolumes showed to be significantly correlated with immunohistochemical staining with the exogenous nitroimidazole derivative pimonidazole and the endogenous hypoxia related marker CA IX.[33] In head and neck xenograft tumors [18F]FMISO also correlated with pimonidazole staining. This correlation depended strongly on the microregional pattern of hypoxia[34] as the distribution on the microscopic level hampers the detection of smaller potentially clinically relevant subvolumes of hypoxic cells.[35] This indicates that caution must be taken when studying smaller tumor (sub)volumes. Several clinical studies have already demonstrated the prognostic value of [18F]FMISO uptake in for example head and neck cancer and lung cancer.[36-38] This may indicate that treatment adaptations directed to hypoxia might be based on [18F]FMISO PET findings.

The prognostic value of [18F]FMISO PET imaging in hypoxia was assessed in a cohort of 20 patients with stage III-IV oropharyngeal carcinomas. Patients underwent two pretreatment [18F]FMISO scans and one during the fourth week of platinum based intensity modulated chemoradiotherapy.[39] Despite the evidence of detectable hypoxia in 18 out of 20 patients an excellent locoregional control was obtained. The presence or absence of hypoxia as defined by [18F]FMISO uptake did not correlate with patient outcome. There were even two patients who had residual detectable hypoxia on the mid treatment scan and they showed excellent tumor control as well. It might be assumed that high dose radiotherapy combined with Cisplatinum, a potent radiosensitizer, overcomes the negative effect of hypoxia in these cases. Contrary to this is the evaluation by Zips *et al.* They studied 25 patients with advanced stage head and neck cancer undergoing four repetitive [18F]FMISO PET scans during a course of radiochemotherapy. Scans were obtained before, at the end of the first two weeks and in the fifth week of treatment. Not baseline uptake, but the [18F]FMISO uptake in the first and second week showed to be of strong prognostic value.[40] Eschmann *et al.* investigated hypoxia with [18F]FMISO PET scans in 26 head and neck cancer patients and 14 non small cell lung cancers before start of radiotherapy. Dynamic and static scans were acquired for which standardized uptake values (SUVs) and signal to background ratios were calculated. These data were correlated with follow-up data concerning local recurrence. They found that an accumulating type curve of [18F]FMISO, a high SUV and high tumor to background ratio at 4 hours after injection were highly suggestive of incomplete response. These results indicate that the outcome after radiotherapy can be predicted on the basis of kinetic behavior of [18F]FMISO in tumor tissue.[38] Rischin *et al.* evaluated the predictive value of [18F]FMISO in a hypoxic imaging side-study of a larger randomized trial comparing chemoradiotherapy and tirapazamine with chemoradiotherapy alone in 45 advanced stage head and neck cancer patients. [18F]FMISO PET imaging was performed at baseline and during treatment at week 4 to 5 identifying 32 hypoxic tumors. The risk of locoregional failure was significantly higher for hypoxic tumors not treated with the hypoxic cytotoxin tirapazamine. Patients without hypoxia and treated with chemotherapy alone had a low risk of locoregional failure demonstrating the need for upfront patient selection in intensified treatment protocols.[36] Furthermore, the study shows the potential to select a group of patients that would possibly be cured with less intensive treatment thereby avoiding an unnecessary increase in side effects and consequential costs.

Although a wide experience with [18F]FMISO has been obtained, some uncertainties regarding its biodistribution and consequential relatively low signal-to-background ratio still remain.[41] Therefore other 2-nitroimidazoles (Table I) like [18F]fluoroazomycin arabinoside ([18F]FAZA) and [18F]fluoroerythronitroimidazole ([18F]FETNIM) were developed and studied.

Like [18F]FMISO, [18F]FAZA undergoes reduction in hypoxic conditions and will be retained in the cell. This tracer has been assessed in an animal tumor model in which mapping of hypoxia with [18F]FAZA was compared to immunohistochemical staining with pimonidazole. In this setting the clinical drawbacks (low resolution of PET and a slow uptake and

TABLE I.—*Some biokinetic features of [18F]FMISO compared to other tracers.*

Tracer	Solubility	Clearance	Uptake ratios
[18F]FMISO [44]	Lipophilic	Relatively slow	Low
[18F]FAZA [44]	More hydrophilic	Faster	Higher
[18F]FETNIM [48]	More hydrophilic	Faster	Higher
[124I]IAZA [47]	More lipophilic	Slower	Lower
[18F]HX4[50]	More hydrophilic	Faster	Similar
[18F]EF5[54]	More lipophilic	No comparison	No comparison
[18F]EF3[51]	More lipophilic	Similar	Similar
[18F]EF1[56]	More hydrophilic	No comparison	No comparison
[64Cu]ATSM[57]	Lipophilic	Faster	No comparison

washout of the tracer) were taken into account. Accurate quantitative maps of tumor hypoxia could be generated using [18F]FAZA PET imaging with excellent spatial agreement after pimonidazole staining.[42]

Based on the chemical properties of [18F]FAZA (*e.g.* more hydrophilic) it was assumed to have more favorable biokinetic characteristics (*e.g.* a faster clearance from the blood) compared to [18F]FMISO. However, clinical data of these two tracers show similar distribution one hour after injection with lower tumor concentration for [18F]FAZA.[43] At that time point the tumor to muscle ratio (TMR) did not differ. Three hours post injection evaluation demonstrated lower [18F]FAZA uptake, indicating lower sensitivity compared to [18F]FMISO. Contrary to these results is the evaluation of [18F]FAZA *versus* [18F]FMISO in an experimental animal model which showed better tumor to background (T/B) ratios for [18F]FAZA.[44] [18F] FAZA was also studied in xenografted and murine tumor bearing mice undergoing hypoxia evaluation by means of pO2-polarography, autoradiography, immunohistochemical staining and PET.[45] [18F]FAZA autoradiography showed good spatial correlation with hypoxic tumor subvolumes based on pimonidazole staining of the same tumor sections. [18F] FAZA was clinically tested in 50 patients with a variety of tumor categories. It proved safe and showed good imaging quality, especially for gliomas.[46]

The iodinated form of the [18F]FAZA tracer, [124I] IAZA, has been compared to [18F]FMISO and [18F] FAZA in a mouse model.[47] The T/B ratios for [124I] IAZA were comparable to those of [18F]FMISO but lower than for [18F]FAZA and therefore less suitable for hypoxia detection compared to [18F]FAZA.

[18F]FETNIM, a more hydrophilic tracer compared to [18F]FMISO, was studied in murine tumors under two different oxygenation conditions.[48] Significant higher tumor-to-muscle radioactivity uptake ratios for atmospheric air breathing mice compared to carbogen breathing mice for both [18F]FMISO and [18F] FETNIM were found indicating that tracer uptake is oxygen dependent. Autoradiographic analysis showed no difference in intratumoral tracer uptake between the two markers. Uptake in normal tissue was, except for muscle tissue under hypoxic conditions, significantly lower for [18F]FETNIM which might result in improved contrast when [18F]FETNIM is used for PET imaging.

Another hypoxic marker that has been evaluated is the 2-nitroimidazole nucleoside analog [18F] HX4 (3-[18F]fluoro-2-(4-((2-nitro-1H-imidazol-1-yl) methyl)-1H-1,2,3,-triazol-1-yl)-propan-1-ol). Dubois *et al.* studied the more hydrophilic [18F]HX4 in a rat rhabdomyosarcoma tumor model. They found that the [18F]HX4 tracer retention time was shorter for normal tissue compared to tumor tissue. This fast decrease in background signal might provide a larger imaging window and a better biostability of [18F]HX4. On the whole-tumor level, they found no significant relationship between [18F]HX4 accumulation and the hypoxic fraction obtained with pimonidazole immunohistochemical staining. However, on the tumor-regional level a strong and significant relationship could be established. Furthermore, a significant increase in tracer accumulation was found in animals breathing 7% oxygen compared to animals exposed to a combination of nicotinamide, to reduce perfusion limited hypoxia, and carbogen.[49] The use of both [18F]HX4 and [18F]FMISO PET scans was evaluated in a small cohort of 12 head and neck cancer patients. Eight tumors showed similar uptake on both [18F]HX4 and [18F]FMISO imaging, two tumors showed only uptake on either [18F]HX4 or [18F] FMISO imaging and two tumors showed no uptake at all. The endogenous hypoxia-related marker CA IX could be studied in nine resected specimens. Based on this small number, [18F]HX4 had a higher sensitivity and specificity compared to [18F]FMISO. Furthermore [18F]HX4 was indeed cleared faster from the blood than [18F]FMISO and had a shorter injection-acquisition time.[50] Further research is needed to establish the value of [18F]HX4 for hypoxia imaging.

The hypoxia tracers [18F]EF3 (2-(2-nitroimidazol-

1-yl)-N-(3,3,3-trifluoropropyl)acetamide) and [18F] EF5 (2-(2-nitro-1[H]-imidazol-1-yl)- N-(2,2,3,3,3-pentafluoropropyl)acetamide) belong to the class of fluorinated etanidazole compounds. The biokinetics of [18F]EF3 have been compared to [18F]FMISO using a mouse model. Both tracers exhibited a more or less similar biokinetic profile, except for the fact that higher uptake was observed for [18F]FMISO in some normal well oxygenated tissue structures.[51] In a rat rhabdomyosarcoma tumor model the quantitative comparison with [18F]FMISO showed no difference in the visualization of hypoxia.[52] Testing [18F]EF3 in 10 head and neck cancer patients, however, proved safe but only one tumor could be visualized.[53] Concerning [18F]EF5, preclinical data showed promising results for hypoxia detection in tumor bearing rats with this radiolabeled tracer.[54] The clinical evaluation for [18F]EF5 in eighteen head and neck tumors showed good potential for [18F]EF5.[55] Similar to [18F]EF5 is the [18F]EF1 tracer that has been evaluated in a rat tumor model showing good hypoxia detecting capacity.[56]

Different from the tracers discussed is [64Cu]-diacetyl-bis(N4-methylthiosemicarbazone) ([64Cu] ATSM). [64Cu]ATSM is not a bioreductive nitroimidazole derivative. It has the ability to accumulate selectively in hypoxic tumors as the low oxygen tension alters the redox environment. Lewis *et al.* investigated the biokinetics *in vitro* and *in vivo* and found fast uptake of [64Cu]ATSM selectively to hypoxic tissue.[57] Interestingly, this marker showed to be invalid in some tumor types, probably due to inter-tumoral differences in retention mechanisms.[58] Moreover caution should be taken when hypoxia measurements are conducted early after tracer administration as O'Donoghue *et al.* reported a discrepancy between [18F]FMISO and [64Cu]ATSM hypoxia measurements at early time points at which [64Cu]ATSM showed a negative correlation with hypoxia.[59] These findings might be explained as follows. First, tumors with a low correlation between [64Cu]ATSM uptake and hypoxia distribution might have different cell line dependent copper reduction pathways causing differences in the balance between the cellular import and export of copper. Second, carbogen improves perfusion around hypoxic cells, which increases the [64Cu]ATSM availability. This in turn might result in more tracer uptake. In this light [64Cu]ATSM might be considered a perfusion marker at early time points. Further characterization of these retention patterns is needed.

[64Cu]ATSM has been clinically tested in 38 patients with cervical cancer undergoing pre-treatment semi-quantitative analysis of [64Cu]ATSM uptake showing a significant inverse relation of [64Cu] ATSM tracer uptake regarding progression free survival and cause specific survival.[60] This approach was also studied in 19 non-small cell lung cancer (NSCLC) patients. The response could be evaluated in 14 patients and showed the mean TMR to be significantly lower in responders compared to non-responders.[61] In another study a semi-quantitative analysis of the pretreatment [64Cu]ATSM PET scan in 17 patients with locally invasive or node positive rectal cancer was performed.[62] Patients treated with neo-adjuvant chemoradiotherapy and surgery of hypoxic tumors showed worse overall and progression free survival relative to patients with better oxygenated tumors. In hypoxic tumors the mean TMR for downstaged tumors was significantly lower compared to non-downstaged tumors, however this difference in mean TMR for these two groups was not statistically significant.

From the aforementioned it can be concluded that several techniques are available as a prognostic instrument for analyzing hypoxic tumors. Of these modalities, PET imaging seems promising for clinical use as it is non-invasive and able to inform about the oxygen status of the tumor as a whole. Further clinical investigation is needed to explore the actual predictive value of hypoxia imaging by means of PET with radiolabeled hypoxic tracers.

Other tracers for tumor characterisation

Not only hypoxia related tracers are available for tumor characterization. Several other PET tracers that give insight into the tumor microenvironment and tumor characteristics are used clinically. Tumor cell proliferation can be quantified by [18F]-fluorothymidine ([18F]FLT) PET and search has been undertaken to define its predictive role in evaluating treatment response at the start or early during treatment.[63] Tumor growth comes with protein synthesis which can be visualized with [11C]-methionine and [18F]-fluoroethyltyrosine ([18F]FET) PET tracers. Most experience with these tracers has been gained in the field of neuro-oncology.[64] Pathways that are associated with inducing and maintaining cancers are influenced by the epidermal growth factor receptor (EGFR)-family receptors, which can be visualized by targeted radionuclide based imaging.[65] Opposed to tumor cell maintenance is cell death. An important

mechanism of cell death induced by radiotherapy is apoptosis. Apoptosis due to cytotoxic therapy can be evaluated by means of radiolabeled tracers such as technetium99m (^{99m}Tc) labeled annexin V.[66] All these tracers have in common that they evaluate a specific property of the tumor itself or the tumor microenvironment. In contrast, [^{18}F]FDG PET reveals glucose metabolism which reflects overall metabolic activity. This metabolic activity is indirect related to proliferative activity and tumor oxygenation as tumor cell proliferation and hypoxia increase glucose metabolism. This will be discussed in more detail later.

Strategies to overcome hypoxia

There are several ways in which treatment adaptation can overcome the negative effect of tumor hypoxia on local tumor control. One approach is characterized by improving the oxygen level in blood plasma and ameliorating the blood perfusion of the tumor. The ARCON concept counteracts hypoxia in two ways. First it thwarts diffusion limited hypoxia by breathing the hyperoxic gas carbogen and second it counteracts perfusion limited hypoxia by means of a vaso-active agent, nicotinamide.[7] Janssens *et al.* applied this concept in a randomized phase III trial for 345 patients with cT2-4 squamous cell laryngeal cancer and found equivalent 5 year local control rates (78% for AR *versus* 79% for ARCON, P=0.80) with improved 5 year regional control rates for patients treated with ARCON (93% *versus* 86%, P=0.04). Improvement of regional control was specifically shown in patients with hypoxic tumors compared to patients with well oxygenated tumors (100% *versus* 55% respectively, P=0.01).[25] Interestingly, in this study hypoxia was quantified based on uptake and binding of the 2-nitroinidazole derivative pimonidazole. These results indicate that modification of the microenvironment, *i.e.* reducing the hypoxic tumor cell population, can improve outcome in patients with hypoxic tumors.

During irradiation the negative effect of hypoxia can also be reduced by the use of agents that mimic oxygen, for example nimorazole. A double blind randomized trial, which studied placebo *versus* nimorazole in primary irradiation of 422 patients with supraglottic carcinoma of the larynx and carcinoma of the pharynx, found a significantly better locoregional control rate for the nimorazole group (49% *versus* 33%, P=0.002). There was no significant effect on overall survival.[5]

Adding the hypoxic cytotoxin tirapazamine to chemoradiotherapy regimens for head and neck cancer patients have showed feasible.[67] As aforementioned, Rischin *et al.* found that tirapazamine additional to chemoradiotherapy for advanced stage head and neck cancer showed significant improved locoregional control rates in patients with hypoxic tumors.[36] The subsequent phase III study randomly assigned 861 patients to be treated with chemoradiotherapy plus tirapazamine or chemoradiotherapy alone. Locoregional control and overall survival did not improve with the addition of tirapazamine.[68] It should be noted that questions remain about the quality of the radiotherapy in this multi center trial. Inferior quality of radiotherapy had a major impact on outcome. However, probably more important is the fact that these patients were not selected for the presence of hypoxia, which might explain equal outcome for both treatment arms. The subgroup of patients bearing more hypoxic tumors can have a potential advantage of hypoxia modification but in this trial the benefit was lost due to the fact that the less hypoxic tumors in the same treatment arm have a diluting effect on outcome data. This indicates once more that pre-treatment selection of patients is of paramount importance in the case of hypoxia modification. Moreover hypoxia modification for less hypoxic tumors is not only a futile effort, it might even potentially harm the patient as treatment related toxicity could increase with hypoxia targeted therapy. This was seen in the aforementioned phase III randomized trial comparing tirapazamine added to chemoradiotherapy for head and neck cancer patients. Acute toxicity like muscle cramps, diarrhea and rash was increased in the group of patients receiving tirapazamine.[68]

Unfortunately, none of the clinical trials studying the effect of hypoxia modification were stratified to the degree of hypoxia what might have contributed to the negative outcomes. Importantly, a meta analysis by Overgaard, showed a clear benefit for hypoxia modification with improved local control rates and overall survival rates in a pooled analysis of 10,108 patients out of 86 randomized trials concerning several different tumor sites.[6]

Reproducibility of hypoxia PET imaging

Another way of counteracting hypoxia in solid tumors is accomplished by increasing the irradiation dose.[1] This higher dose will eradicate more clono-

genic cells and thus may improve local control. The introduction of Intensity-Modulated Radiation Therapy (IMRT) made it technical possible to increase the dose to subvolumes within the tumor.[69]

The question remains whether or not the hypoxic areas as visualized by a single pre-treatment PET scan remain stable over time so this information can be used to direct a higher dose of irradiation to these subvolumes over a course of fractionated radiotherapy lasting several weeks. Various attempts have been made to answer this question, as concerns may arise about the reproducibility of hypoxic tumor areas, as hypoxia is a dynamic process. The reproducibility of [18F]FMISO imaging was tested in 13 head and neck cancer patients undergoing 2 sequential [18F]FMISO PET scans three days apart. Tracer distribution was analyzed on a voxel-by voxel basis. Considering the entire tumor volume, strong correlation was found in 71% of patients. However, this percentage was reduced to 46% when the hypoxic subvolume was analyzed. This finding is possibly due to the amount of acute, perfusion limited, hypoxia that fluctuates over time.[70] The consequences of these findings for the dose distribution of the IMRT dose escalated treatment plans were evaluated for seven of these patients. The spatial distribution of hypoxia changed, compromising the coverage of the irradiation boost to the hypoxic tumor volume.[71] Contrary to this are the results of a recent study with repetitive [18F]FMISO imaging in 11 patients with head and neck cancer. The quantitative evaluation of the two repetitive PET scans performed were highly reproducible.[72] The evaluation parameters proved to be similar for the two scans. The tumor area with maximum uptake on the first scan was slightly different from that on the second scan, with a mean difference of 4.3 mm (range 0.9-5.7 excluding one outlier of 11.8mm). These findings indicate that, although hypoxia is in part considered a dynamic phenomenon, some tumor areas have a near stable degree and localization of hypoxia. The conflicting results can be explained by the small number of patients evaluated in each trial. Furthermore, there are several discrepancies regarding the setup and acquisition protocol of the scans performed. For example different time intervals were used between [18F]FMISO administration and scanning (117-195 minutes *vs.* 262±21 minutes) and the T/B ratio threshold applied was different (≥1.2 *vs.* ≥1.5). Moreover, the conflicting outcome can also be the result of differences in imaging acquisition protocols (2-dimensional *versus* 3-dimensional) resulting in differences in image contrast and statistical noise.

Recently, the reproducibility of [18F]FAZA imaging has been studied in tumor bearing mice during fractionated radiotherapy. The investigators found that these hypoxia PET scans were able to represent tumor hypoxia in a quantitatively correct way and that the PET scans were highly reproducible.[73]

Further research is needed to establish an optimal scanning protocol which enables the radiation oncologist to direct a higher radiation dose to these PET based hypoxic tumor areas.

The selective boosting of tumor areas has proved feasible in a study in which [18F]FAZA PET revealed hypoxic subvolumes in 11 out 18 patients with advanced head and neck cancer. The authors suggested a dose escalation to 80.5 Gy for these hypoxic subvolumes.[74] Lee *et al.* performed a feasibility study determining hypoxic subvolumes in head and neck cancer patients by means of [18F]FMISO PET. It was possible to escalate the irradiation dose up to 84 Gy for all ten patients, without exceeding normal tissue constraints.[75] This approach, using [18F]FMISO, showed feasible in 2 other planning studies as well.[76, 77] This principle was also tested for hypoxic subvolumes using [64Cu]ATSM PET. The investigators found it possible to demarcate a tumor subvolume harbouring hypoxia and escalate irradiation dose.[78] Abovementioned dose escalating approaches seem promising, however, clinical trials are needed to evaluate the gain in local tumor control for tumors with hypoxic characteristics.

FDG PET and hypoxia

Significant clinical experience has been gained and research has been done with the [18F]FDG tracer. Nowadays, it is a widely used tracer for staging and therapy response evaluation in oncology.[79] In contrast to hypoxia tracers, [18F]FDG PET visualizes glucose uptake and thus provides information on the metabolic state of the tumor. Under physiological circumstances non-tumor cells metabolize glucose to pyruvate, which in turn is oxidized in the mitochondria to form adenosine triphosphate (ATP). This so-called oxidative phosphorylation is highly dependent on the presence of oxygen. As cells become oxygen deprived the process of oxidative phosphorylation hampers, which results in an increase in glycolytic flux because of anaerobic

glycolysis. This is called the Pasteur effect.[80] From this it can be hypothesized that increased [18F]FDG uptake reflects the increased glycolytic flux in anaerobic circumstances. In this light [18F]FDG might be considered a marker of hypoxia. This concept was studied in a pre-clinical study evaluating [18F]FMISO and [18F]FDG uptake in tumor bearing mice. It was concluded that [18F]FMISO might be able to identify hypoxia whereas this was unlikely for [18F]FDG.[81] Rajendran *et al.* evaluated the possible role of [18F]FDG as a hypoxia marker in 49 patients suffering from head and neck cancer (26 patients), soft tissue sarcoma (11 patients), breast cancer (7 patients) and glioblastoma multiforme (5 patients). The pre-treatment [18F]FDG PET and [18F]FMISO PET scans were evaluated. Comparison of whole tumor images showed a small but significant correlation between [18F]FDG uptake and the hypoxic volume, especially for head and neck tumors. There was, however, no correlation when the tumor types were analyzed separately indicating a wide variation in the relationship of hypoxia and metabolism as visualized by [18F]FMISO and [18F]FDG.[82] The correlation between [18F]FDG, [18F]FMISO and pO(2)-polarography has been studied in 24 patients with head and neck cancer. Despite a moderate correlation of the standardized uptake value of [18F]FDG with the T/B ratio of [18F]FMISO, the uptake of the [18F]FDG tracer did not correlate with the results of pO(2)-polarography.[83] Another tracer, [14C]EF3, has been tested in this setting in tumor bearing mice. Both [18F]FDG and [14C]EF3 were administered after which PET imaging and autoradiography were performed. A low correspondence was shown between the two tracers as the computed matching indices were low.[84] The hypoxia specific tracer [18F]FAZA has been compared to [18F]FDG in four carcinoma cell lines. An excellent display of hypoxia by [18F]FAZA was found while [18F]FDG did not show high specificity for hypoxia.[85] It can be concluded that several attempts have been made to correlate [18F]FDG uptake to hypoxia, however up to now none have indicated that [18F]FDG is a specific tracer for hypoxia. This might be explained by the mechanisms malignant cells use to fulfill in their energy demands. Malignant cells do only to a limited extend generate ATP by means of anaerobic glycolysis (Pasteur effect), they mainly generate energy by means of aerobic glycolysis. This is called the Warburg effect [86] and indicates glycolytic ATP production under normoxic conditions. It is the aerobic glycolysis that causes high [18F]FDG uptake as is visualized on PET. This implies that the glycolytic flux, which is visualized by [18F]FDG uptake, is only to a limited extend revealing hypoxic conditions (Pasteur effect) because of the generally enhanced glycolysis under aerobic conditions (Warburg effect) in tumors.

Although [18F]FDG is not a hypoxia tracer, it is of value as its accumulation reflects overall metabolic activity and tumor load. For this reason targeting tumor areas with an increased metabolic burden might be beneficial in tumor eradication.

The metabolic tumor burden on [18F]FDG PET measured at baseline for NSCLC patients has shown to be of prognostic value. For 169 patients diagnosed with NSCLC the pre-treatment [18F]FDG PET scans were reviewed retrospectively. The Metabolic Tumor Volume (MTV) and Total Lesion Glycolysis (TLG) at baseline of primary tumor and involved lymph nodes showed prognostic significance independent of clinical stage. These results suggest that MTV and TLG are better prognostic measures than SUVmax and SUVmean.[87] In 61 patients with NSCLC, the staging [18F]FDG PET scan was analyzed with regard to MTV and treatment outcome. In a subgroup of 39 patients treated with definitive intent, multivariate analysis showed an independent significant prognostic value of MTV for overall survival and a trend for progression free survival.[88] A study in 51 inoperable NSCLC patients showed that the SUVmax was a prognostic factor for disease specific survival and overall survival and predictive for tumor response.[89] Similar results have been reported for several other tumor sites including head and neck cancer[90] and esophageal cancer[91].

A major advantage of PET imaging is the ability to monitor treatment effects over time, and by doing so, to modify and optimize treatment. Clinical data have shown the potential role of [18F]FDG PET in providing insight in tumor response to therapy.[92] Sequential imaging during treatment by means of [18F]FDG PET reveals glucose uptake patterns that might predict treatment outcome. Hentschel *et al.* evaluated repeated [18F]FDG PET scans during chemoradiotherapy for 37 patients with head and neck cancer. One scan was performed before start of treatment and 3 scans were obtained during treatment. The two year overall survival and locoregional control rates were significantly better if the SUVmax of the primary tumor decreased 50% or more in the first two weeks of treatment.[93] Another prospective trial evaluating the prospective value of [18F]FDG PET in 77 head and neck cancer

patients showed that only the method of visual interpretation of [18F]FDG uptake could predict treatment outcome in patients with oral cavity or oropharyngeal carcinoma.[94] The authors suggest that for head and neck cancer further research is needed regarding the predictive role of [18F]FDG PET imaging during treatment as several studies have been undertaken with inconsistent outcomes.

For 23 patients with inoperable NSCLC the metabolic changes during cytotoxic treatment have been studied by means of repeated [18F]FDG PET scans before, during and after treatment with accelerated radiotherapy.[95] The evolution of SUVmax was subject to large intra-individual variation. All 23 patients showed a non significant increase in the first week of radiotherapy whereas in the second week and at the end of treatment a significant decrease in SUVmax was observed. No change during therapy was observed for responders while non responders showed a significant 48% increase in the first week and a significant 15% decrease in the second week. These findings illustrate the need for treatment adaptation as [18F]FDG PET reveals different metabolic responses reflecting different tumor characteristics. During treatment [18F]FDG PET evaluation was also studied by Usmanij *et al.* [18F]FDG PET scans were performed pre-treatment, at the end of the second week of treatment and two weeks and three months after completion of chemoradiation in 28 patients with locally advanced NSCLC. Progression free survival showed to be significantly longer with a decrease in total lesion glycolysis of 38% or more as seen on the PET at the end of the second week of treatment.[96] Recent studies have shown similar results for patients with rectal cancer[97], esophageal cancer,[98] breast cancer [99] and cervical cancer.[100] These findings indicate that it is possible to select patients prior to treatment and also early during treatment for an individualized treatment approach based on [18F]FDG PET imaging.

Conclusions

Several tumor related microenvironmental factors are known to have a negative influence on the outcome of cytotoxic treatment. Hypoxic tumors are known to be more radioresistant which results in lower disease control rates. Measurements by oxygen electrodes or immunohistochemical staining are invasive and are not able to give a longitudinal representation of the oxygen status of a tumor. Functional imaging by means of PET with hypoxia tracers such as [18F]FMISO and [18F]FAZA has the advantage that it is non-invasive and that it visualizes the tumor as a whole. PET imaging informs the clinician about the oxygenation status and the geographical distribution of tumor hypoxia. In case of hypoxia, the cytotoxic treatment can be optimized in an attempt to overcome the negative effect of tumor hypoxia. With this aim, several approaches (*e.g.* the use of oxygen mimickers and ARCON) have been studied. These have proven beneficial, however upfront patient selection has shown to be of paramount importance. [18F]FDG is another tracer that displayed prognostic value in several solid tumor types studied. Because of the Warburg effect it is not a specific hypoxia tracer, however it is still valuable for treatment decision making as increased [18F]FDG uptake is indicative of increased cell proliferation and glycolysis.

Visualization of treatment resistant tumor subvolumes based on PET imaging allows individualization of treatment and specific targeting of these subvolumes. PET imaging seems promising for this purpose although uncertainties remain regarding its reproducibility. Once this has been clarified, functional PET imaging gives the opportunity to adapt radiation dose according to the specific microenvironmental circumstances. This might optimize local control and reduce toxicity.

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