As the metabolic microenvironment markedly influences the therapeutic response of malignant tumors, imaging of the microenvironment is one of the goals researchers have been aiming at for years. Several methods, such as positron emission tomography, functional magnetic resonance imaging (MRI) or contrast enhanced MRI/CT, are now available. For radiation oncology, tumor oxygenation and perfusion are the most important (patho-) physiological parameters that might be included in radiotherapy regimens and treatment planning. In order to overcome resistance of tumor cells resulting from hypoxia, positron emission tomography (PET) using nitroimidazole tracers is the most advanced technique at this time. Since reproducibility of the PET signal/tracer distribution, thresholding and exact quantification are not thoroughly understood and further investigation is needed before including it into radiotherapy regimens. To image tumor perfusion, dynamic contrast enhanced computed tomography (DCE-CT) or dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) are the most suitable techniques. Co-investigation of tumor oxygenation and perfusion should be performed in order to investigate their interaction and consequences for radiooncology.

**Key Words:** tumor physiology, hypoxia, perfusion, biological imaging, dose painting, radiotherapy.

**Abbreviations used:** 18F-FDG — 18F-fluorodeoxyglucose; FLT — 3´-deoxy-3´-[18F]-fluorothymidine; FMISO — 18F-fluoromisonidazole; FAZA — 18F-azomycinarabinoside; 15O-H2O — 15O-labelled water; BOLD MRI — blood oxygen level-dependent magnetic resonance imaging; RGD — arginine-glycine-aspartic acid; SPECT — single photon emission computed tomography; SUV — standardized uptake value; TPZ — Tirapazamine.

**Table. Modern imaging to visualize tumor (patho-) physiology**

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Oxygenation</th>
<th>Perfusion</th>
<th>Angiogenesis</th>
<th>Cell density</th>
<th>Glucose uptake</th>
<th>Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET Nitroimidazoles (FAZA, FMISO...)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET FLT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET FDG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET Annexin V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET Water, Inert gas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET Dynamic acquisition, 15O-H2O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI DCE-MRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI BOLD-MRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI Diffusion weighted imaging</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI MRS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT DCE-CT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As such, technological developments in the last decades have focussed on improvements in hardware and software systems, able to deliver irradiation with a high geometrical accuracy. Techniques such as stereotactic fractionated radiotherapy, radiosurgery, IMRT and three-dimensional (3D) planned brachytherapy are characterised by a high level of accuracy in the delivery of irradiation to tumor tissue, sparing the surrounding normal structures and leading to a substantial improvement in treatment results. In 2000, Ling et al. [4]
proposed the concepts of biological target volume and multidimensional conformal radiotherapy, which enable dose distribution to be adapted to both, the morphology and the biology of the tumor. Using methods to image tumor (patho-) physiology, a biological pathway with special significance for tumor response to the treatment could become the target of radiation therapy.

**Imaging of hypoxia.** The two main physiological characteristics of the microenvironment in tumors which impact on therapeutic outcome are oxygenation and pH. As the oxygen tension is reduced below 10–15 mmHg cells become increasingly resistant to radiation damage. Anoxic cells are approximately three times more resistant to radiation than those irradiated under normoxic conditions. Because of their potential importance for treatment outcome, large efforts have been made over the years to identify strategies that will reduce or eliminate hypoxic cells within tumors. Such approaches are breathing high oxygen content gases during radiation treatment under normobaric or hyperbaric conditions, anemia correction or improving tumor blood supply through vasoactive drugs to increase oxygen availability, the use of chemical radiosensitizers which could «fix» radiation damage in a manner similar to oxygen or killing the resistant cell population either by drugs or by enhanced radiation dose.

Finding adequate imaging methods for the assessment of hypoxia in cancer patients is still a challenge for years. The use of PET is attractive because it provides a non-invasive, repeatable whole tumor assessment in vivo. Hypoxia tracers should fulfill several conditions: (1) to be captured specifically by hypoxic cells using an oxygen-specific retention mechanism; (2) to be sufficiently delivered to a perfusion–limited microenvironment; (3) to produce a low level of non-specific metabolites, and (4) labelled metabolites of hypoxia tracers should not be found in the circulation at the time of imaging. None of the currently available hypoxia tracers available do perfectly meet all these requirements. From the number of PET probes developed for measuring hypoxia, 18F-fluoromisonidazole (18F-FMISO) is the most widely studied. The early delivery of 18F-FMISO into tumors correlates with blood flow, and its high lipophilicity enables flow-independent measurements of tissue hypoxia 90–120 min following injection. Applying static PET examinations, tumor uptake is generally quantified relative to plasma or muscle levels. Typically, a tumor to blood ratio of ≥1.2 has been used as a reasonable cut off point between normoxia and hypoxia for 18F-FMISO. Tumor to muscle ratio of 18F-FMISO do significantly correlate with tumor hypoxic fraction as measured by the polarographic needle electrode in 16 head and neck cancer (HNC) patients [5]. PET measurements of hypoxia have been used to show the presence of hypoxia in several solid tumors. In 83% of our patients with human head and neck cancer hypoxic subvolumes were detected. Two patterns of hypoxic subvolumes were described: In 61% of the patients the hypoxic area was located in a single confluent area and in 22% hypoxic subvolumes were diffusely dispersed [6].

Some studies report associations between PET measurements of hypoxia and radiotherapy outcome [7]. In 45 patients with pretreatment FMISO PET imaging, hypoxic tumors were less likely to fail when treated with a combined regimen of chemoradiation and the hypoxic cytotoxic agent Tirapazamine (TPZ) when compared to a non-TPZ regimen [8]. Serial 18F-FMISO imaging at 3–4 weeks within the radiation course for head and neck cancer have been performed with varying results. In the study by Eschmann et al. [9], kinetic curve types representing tissue hypoxia were defined. Change of curve type at 30 Gy was correlated with patient outcome. In one prospective study with 20 patients, neither the presence nor the absence of hypoxia, as defined by positive 18F-FMISO findings on the mid-treatment PET scan, correlated with patient outcome [10].

To address a potential problem that severely hypoxic/necrotic tissues show low uptake of a PET tracer and are ignored when using standardized uptake values (SUV), kinetic models for the analysis of dynamic 18F-FMISO-PET and FFA-PET data were described and evaluated [11, 12]. Models are used to derive parameters that reflect well oxygenated/perfused tissue, diffusion-limited hypoxia, perfusion-limited hypoxia and severe hypoxia/necrosis.

Despite this large progress made in hypoxia imaging, the reproducibility of intratumor distribution of hypoxia PET tracers is a challenge. The premise of a radiotherapy concept including dose escalation requires that hypoxic regions in the tumor remain relatively stable before and during the course of IMRT treatment over several weeks. Temporal changes in the distribution of the hypoxia signal and their relevance for dose painting treatment plans have been reported [13, 14].

Besides PET there are MRI based methods to image oxygenation. Blood oxygen level-dependent magnetic resonance imaging (BOLD MRI) is based on the fraction of paramagnetic deoxyhemoglobin and diamagnetic oxyhemoglobin and the effect of the latter on MR signals [15]. Primarily BOLD-MRI was developed for the assessment of intravascular blood oxygenation. However, recent data show that it may also allow an estimation of tissue oxygenation [16]. Although BOLD MRI does not require injection of an exogenous contrast agent, its signal can be influenced by factors other than hypoxia including blood flow, CO2 tension, hematocrit, pH and bisphosphoglycerate. In a recent study of 24 patients with prostate cancer undergoing radical prostatectomy, BOLD MRI was performed preoperatively and correlated with pimonidazole staining. R2* (MR relaxivity parameter) maps from BOLD MRI yielded high sensitivity but low specificity for defining hypoxic tumor regions stained with pimonidazole [16]. Comparing BOLD-MRI with oxygen electrode measurements, a correlation was observed between R2* and the hypoxic fraction <5 mm Hg (HP5) and a trend was noted between R2* and pO2 [17].

**Imaging of tumor perfusion.** Hypoxia is a key factor in a vicious circle driving tumor growth [18, 19]. On the one hand it is a consequence of insufficient vascularization in rapidly growing tumors. On the other hand hypoxia is an important factor driving tumorigenesis and is a key stimulator of angiogenesis. Tumor vessels have, inter alia, blind endings, irregular branching, increased per-
meability and lack of supporting pericytes, are elongated, dilated and tortuous [20]. These features lead to erratic and inadequate perfusion. Hypoxia, therefore, stimulates both angiogenesis and blood flow in tumors but occurs as a result of ineffective tumor vasculature that develops. These «bipolar» phenomena are confirmed in patient studies that did not find any correlation between perfusion parameters and histologically determined microvessel density. The role of perfusion for risk stratification of tumors is controversial as in the field of anti-angiogenesis drug development, reduced blood flow is considered as a response to successful anti-angiogenic therapy whereas in other forms of tumor therapy high perfusion is supposed to be correlated with better outcome. These features highlight the need to increase our understanding of the complex inter-relationships between blood flow, hypoxia and angiogenesis in tumors.

Dynamic contrast-enhanced CT or MRI (DCE-CT, DCE-MRI) provide information about tissue perfusion. Such techniques have been applied to many different tumors. Our own studies in deep pelvic and abdominal tumors showed good correlations between the measurement values obtained by thermal clearance method, dynamic CT and dynamic MRI [21]. Measurement results are related to microvascular density and permeability. Moreover perfusion can be quantified by the introduction of kinetic modelling. The models finally lead to quantification of regional blood volume, regional blood flow and mean transit time. It has been demonstrated that the amplitude of the signal correlates with the microvascular density of the tumor whereas the exchange rate points towards an increased permeability of the tumour vasculature [22]. DCE-CT and DCE-MRI studies have shown an inverse relationship between perfusion and outcome following radiotherapy [23, 24]. It has also been shown, that perfusion MRI is capable of predicting outcome in patients with cerebral metastases who have undergone stereotactic radiotherapy [25]. Moreover, tumor blood supply parameters derived from DCE-CT seem to be useful to characterize tumor vascularization before radiotherapy in patients with non-small-cell lung cancer (NSCLC) [26].

There are also a number of PET probes available for imaging tissue perfusion. From those 15O-labelled water (15O-H2O) is a well validated standard and has been used widely on brain and cardiac PET studies for non cancerous disease. Its short half-life (minutes) makes it attractive for the use in dual scanning studies. Interestingly, a dual tracer PET scanning study using 15O-H2O and 15O-FMISO in brain tumors showed hypoxia in both well and poorly perfused regions of tumors [27]. This observation suggests that at a regional level the presence of hypoxia may be independent of the level of perfusion. On the contrary, a very recently performed study on 13 patients with HNC found a strong negative correlation between perfusion measured by DCE-MRI and hypoxia as measured by static 15O-FMISO-PET [28].

Further studies should explore the exact relationship between perfusion and hypoxia within tumors, how the patterns relate to the prognosis of patients undergoing radiotherapy and how features associated with bad prognosis can be influenced by therapeutic agents or irradiation. Imaging of tumor metabolism. Due to increased anaerobic glycolysis as a result of increased proliferation and hypoxia, tumors exhibit enhanced glucose transport (GLUT-1 activity) which can be imaged by 18F-fluorodeoxyglucose (18F-FDG) PET-tracer uptake. Correlations between FDG uptake and hypoxia as well as proliferation have been shown in the literature. Nevertheless, FDG-PET must not be used as imaging method for hypoxia or proliferation. FDG-PET has mainly been used as a method for tumor staging or search for unknown primaries. As FDG-PET can provide information if cells are metabolically active, it might also be of interest in performing dual or multiple probe PETs to assess tumor (patho-) physiology. For example hypoxic regions with high FDG-uptake could be more resistant to treatment than hypoxic cells with low FDG-uptake.

MR spectroscopy (MRS) allows demonstrating shifts in the distribution of certain metabolites, therefore providing metabolic information about tumour cells and the surrounding tissue. For example the loss of citrate or an increase in the choline/citrate ratio is an indicator for prostate cancer. Other clinical indication for this technique are the diagnosis of malignant brain tumors where the metabolites of potential interest are choline (Cho), creatine (Cr), N-acetyl aspartate (NAA), lipids and lactate. Necrosis can be imaged by demonstration of lipid peaks.

In summary metabolic features are not understood well enough to include this information in radiotherapy treatment planning.

PET imaging of proliferation. For the measurement of proliferation a number of PET tracers are under investigation. Examples are 18F-FLT (3′-deoxy-3′-fluorothymidine), 18F-FMAU (1-(2′-deoxy-2′-fluoro-beta-D-arabinofuranosyl) thymine) and 11C-thymidine, each having its advantages and disadvantages. 18F-FLT accumulation in human tumors has been shown to correlate with tumor cell proliferation as assessed by the Ki-67 labeling index. It is retained in proliferating tissues primarily through the enzyme thymidine kinase [29]. At present the data from the literature show that 18F-FLT-PET appears as a useful tool for early response assessment of tumors. There are no data regarding the integration of 18F-FLT-PET into radiotherapy treatment planning. But if clinical and experimental studies confirm the hypothesis that 18F-FLT has a high sensitivity and specificity for proliferating tissue. This investigation could play an important role in the development of new image-based dose distribution and treatment fractionation strategies.

PET imaging of apoptosis. An increased understanding of the apoptotic process would help to develop strategies to increase or restore the capacity of cancer cells to undergo apoptosis, thus improving cancer therapy. An early sign after initiation of the apoptotic signaling pathway is externalisation of phosphatidylserine from the inner leaflet to the outer leaflet of the cell membrane, followed by membrane «blebbing» and DNA degradation. Annexin V allows detecting cells that undergo apoptosis. Radiolabeling of Annexin V has already
been achieved and animal PET studies are underway. Therefore PET methodology using radiolabeled Annexin V for measuring apoptosis might be a promising tool for detecting apoptosis within patient tumors.

CONCLUSION

Modern imaging methods offer the opportunity to visualize (patho-) physiological pathways with high impact upon radiation treatment response such as tumor hypoxia or perfusion. In addition they have the potential to increase our biological understanding of tumors, providing the basis for the development of new treatment concepts. The (patho-) physiological information derived from PET, MRI or CT could be used to specifically target radiation resistant tumor areas, consequently realizing the concept of biological-based dose painting. However, validation of the imaging results is a long way off and needs further experimental and clinical studies.

REFERENCES