PET is a nuclear medicine imaging technique that can be used to localize, and assess the extent of, specific biological processes within the body. For this purpose, molecules known to be involved in the process of interest are “labeled” with positron emitting radionuclides. After this “tracer” is injected intravenously, it will be distributed throughout the body by the blood circulation. Ideally, the tracer will then accumulate (or bind) specifically at locations where the extent of the biological process of interest is high. The radiation emitted by the radioactive labels is registered with a PET system and reconstructed into a 3D image. This 3D image will reflect tracer activity concentrations, and thereby tracer concentrations, throughout the body. Since the introduction of PET in 1977 and its successful implementation in the clinic over the years, more and more novel PET tracers aimed at imaging a variety of processes are becoming available. After successful preclinical evaluation, tracers are evaluated clinically before being introduced in a routine clinical setting. Technically, PET is capable of accurate quantification of activity concentrations. However, several factors need to be taken into account before the measured activity concentrations can be translated into clinical decisions. A simple example is the total activity injected: this will inherently affect the activity concentrations in tissue, irrespective of the extent of the process of interest. The first step towards translation is relating measured activity concentrations to tracer uptake that is caused solely by the process of interest (“specific uptake”). This can be achieved with full pharmacokinetic modeling using an elaborate imaging protocol. For a tracer to be successfully applied in a routine clinical setting, a simpler imaging protocol and analysis method, such as SUV, is preferred. This thesis aims to validate clinically feasible methods for quantification of tracer uptake for several oncology PET tracers using full pharmacokinetic modeling as reference.
6.1 \([^{18}F]FCH\) PET imaging

**Summary and discussion**

Chapter 1 describes a clinical study to assess the kinetics of \([^{18}F]FCH\). Radiolabeled choline has been introduced as a promising tracer for PCa \([10, 18, 207]\). The increased choline transport and overexpression of choline kinase in PCa is presumed to lead to increased trapping of choline within the tumor cell membrane. With its active transport and specific uptake mechanism, \([^{18}F]FCH\) seems ideal for imaging PCa. However, the results presented in chapter 1 show that quantification of \([^{18}F]FCH\) uptake is not as straightforward. Dynamic PET/CT imaging and arterial blood sampling was performed for eight patients with metastasized PCa. Full pharmacokinetic modeling found \(2T3k+V_B\) to best fit the dynamic PET data from metastatic lymph nodes, but kinetics were too fast for the model to precisely distinguish between \(K_1\) and \(k_3\). The \(1T1k+V_B\) model was found to yield equivalent quantification parameter values, whilst providing more robust fits to the data. SUV showed poor correlation to \(K_1\) derived with \(1T1k+V_B\) full pharmacokinetic modeling (\(R^2<0.34\)). This is likely caused by differences between patients in tissue distribution and clearance of the tracer. Therefore, an alternative simplified method was proposed: normalizing lymph node activity concentrations to the area under the blood TAC (\(SUV_{AUC,WB}\)). The protocol for obtaining this measure would still be feasible in a routine clinical setting and consists of a 30 min PET scan over the aortic arch (started at the moment of injection) followed by a PET scan over the lesions of interest. \(SUV_{AUC,WB}\) yielded better correlation to \(K_1\) (\(R^2 = 0.65\)).

However, like SUV, \(SUV_{AUC,WB}\) does not take into account breakdown of the tracer into metabolites. PET imaging cannot distinguish between signals originating from the tracer and radiolabeled metabolites. As metabolites often exhibit different kinetics than the original tracer, the plasma input function is corrected for the presence of radiolabeled metabolites. To this end, manual arterial blood samples obtained at several time points p.i. were analyzed with HPLC. \([^{18}F]FCH\) metabolite formation rates were found to be very high and large relative differences between patients were observed. Using the alternative simplified method, but this time incorporating metabolite correction (\(SUV_{AUC,PP}\)), yielded high correlation to \(K_1\) (\(R^2 = 0.92\)).

**Outlook**

As \([^{18}F]FCH\) kinetics were found to be irreversible, a direct relation between tracer accumulation and tissue perfusion is expected. Whether this could lead
to clinically relevant quantification errors, should be investigated. Furthermore, $\text{SUV}_{\text{AUC,PP}}$ requires arterial blood sampling because measures from venous blood samples were found to be unreliable. This will limit clinical applicability of the method. Future research should determine whether the performance of $\text{SUV}_{\text{AUC,WB}}$ would be sufficient for clinical applications. If a choline derivative could be found that is not metabolized as quickly as $[^{18}\text{F}]\text{FCH}$, the performance of $\text{SUV}_{\text{AUC,WB}}$ is expected to improve. Currently, studies on development and performance of alternative choline-derivatives are ongoing [30, 31].

In the study presented in Chapter 1, only lymph nodes characterized as malignant were considered. In clinical studies, $[^{18}\text{F}]\text{FCH}$ has been shown to accumulate in benign lymph nodes as well. The inability to distinguish between benign and malignant lymph nodes could be an important limitation of $[^{18}\text{F}]\text{FCH}$ PET imaging. Recently, Oprea-Lager et al. have shown that relative differences in $[^{18}\text{F}]\text{FCH}$ uptake between 2 and 30 min p.i. may characterize lesions as malignant or benign [20]. This indicates that $[^{18}\text{F}]\text{FCH}$ kinetics in benign lesions may fit a different model and this warrants further investigation using pharmacokinetic modeling. Blood sampling may not be required in this case, as intra-patient differences in kinetics would be studied.

Other PET tracers are currently under evaluation: $[^{11}\text{C}]\text{acetate}$ has an uptake mechanism in the tumor cell membranes similar to $[^{18}\text{F}]\text{FCH}$ but requires an on-site cyclotron [208]; anti-$[^{18}\text{F}]\text{FACBC}$, an aminoacid analogue, shows equally fast kinetics whilst, in contrast to $[^{18}\text{F}]\text{FCH}$, being very robust in human subjects. Uptake in tumor tissue, however, was shown to be reversible [209]. Recently, another type of PET tracer for imaging PCa has been gaining interest: prostate-specific membrane antigen (PSMA) targeted PET tracers. PSMA is a dimeric type II integral membrane glycoprotein with high expression on PCa cells, and is associated with disease progression. PMSA PET tracers could potentially image PCa with very high specificity [210]. Given the experience with $[^{18}\text{F}]\text{FCH}$, it would be recommended to perform a full pharmacokinetic modeling study in patients for any novel tracer that is to be introduced in a clinical (research) setting. This way the assumptions made by commonly used quantification methods can be verified before a lot of time, effort and money is invested in clinical trials based on static PET imaging alone.

### 6.2 Hypoxia imaging

#### Summary and discussion

As described in chapters 3 and 5, kinetic modeling of $[^{18}\text{F}]\text{FAZA}$ and $[^{18}\text{F}]\text{HX4}$ is more straightforward and a number of simplified measures were found to
perform well. Also for the most evaluated hypoxia tracer, $^{18}$FFMISO, Wang et al. found simplified methods to be suitable for quantification of tracer uptake [86]. When it comes to quantification of hypoxia with PET, the challenge lies in correctly interpreting of quantification measures, i.e. the translation of “tracer uptake” into “level of hypoxia”. This is complex due to the almost uniform distribution of the tracer throughout the body, the inevitable dependence on perfusion and the heterogeneous distribution of hypoxia in tumor tissue.

$^{18}$F]FAZA, $^{18}$F]HX4 and $^{18}$F]FMISO are nitroimidazole based hypoxia PET tracers. Upon entering the cell the tracer molecule undergoes electron reduction and subsequently either reacts with a macromolecule within the cell and is retained (under hypoxic conditions), or reacts with oxygen and is free to diffuse out of the cell [84]. Herein lies the problem: transport into the cell is not specific to hypoxic cells. In fact, tracer uptake is seen in virtually all tissues. It is only at later time points, when the tracer has had time to clear from normoxic cells, that tracer retained in hypoxic tissue can be visualized with PET. To complicate matters further, tracer delivery to the tissue will depend on perfusion, while hypoxia itself can be caused by a localized (momentary or long term) lack of perfusion [211], and consequently in areas of limited tracer delivery. These considerations should be taken into account when attempting to quantify hypoxia with PET imaging.

Chapter 2 provided an overview of the current status (up to January 1st 2014) of research on hypoxia and perfusion PET imaging in lung cancer. Most suitable tracer for imaging perfusion is $^{15}$O]H$_2$O as for this tracer, due to its 100% extraction, perfusion is equal to K$_1$ (as evaluated with pharmacokinetic modeling, for example using a basis function method). In lung cancer, $^{15}$O]H$_2$O imaging has been thoroughly investigated, but limited data are available on hypoxia imaging. Drawing conclusions from published clinical data is complicated as studies use different hypoxia tracers, different imaging protocols and various simplified parameters for quantification. Pharmacokinetic modeling is a more objective method for quantification of tracer uptake: quantification is based on the shape of TAC instead of its value at one time point p.i. only, and incorporates information on tracer delivery over time. Therefore, pharmacokinetic modeling is often recommended for quantification of hypoxia tracer uptake [212, 213, 214, 215]. Chapters 3, 4 and 5 presented pharmacokinetic modeling results for hypoxia tracers $^{18}$F]FAZA and $^{18}$F]HX4.

In chapter 3, 70 min dynamic PET data, in combination with continuous arterial blood sampling, obtained from nine patients were used to study $^{18}$F]FAZA kinetics in NSCLC. Results indicated a 2T4k+V$_B$ model for $^{18}$F]FAZA kinetics in tumor tissue. In addition, it was found that arterial blood sampling may not be required: the input function can be derived from the PET image
itself and the necessary correction factors can be obtained from venous blood samples. Because variability between patients in both plasma-to-blood ratios and parent fractions was minimal, population based corrections of the input function might be sufficient. This would obviate the need for blood sampling altogether, but these hypotheses should be tested in a larger study population. Good correlation was found between simplified methods and $V_T$ derived with full pharmacokinetic modeling, with best results obtained with TBr ($R^2 = 0.87$, 50-60 min p.i.).

NLR, the method used in chapter 3 for fitting the TAC, is very sensitivity to noise. It can therefore only be used for averaged TAC obtained from VOI large enough to average out noise levels to an acceptable level. However, as the level of hypoxia will likely vary within tumor tissue, differences in tracer uptake may also be averaged out by using large VOI. In chapter 4, several methods for pharmacokinetic modeling at the voxel level were tested by comparing results to NLR. Using the input parameter settings recommended in chapter 4, both Logan graphical analysis and spectral analysis performed well ($R^2 = 0.88$, ICC = 0.93; and $R^2 = 0.79$, ICC = 0.81, respectively). In addition, the performance of TBr was re-evaluated for the smaller VOI used in this chapter. Consistent with the results obtained in chapter 3, good correlation was found for TBr compared with NLR ($R^2 = 0.85$; ICC = 0.80). It should be noted that bias was observed at high $V_T$. Furthermore, VOI cannot be adjusted to exclude high $V_B$ areas based on static PET imaging alone. Further studies are needed to ascertain whether the observed bias and lack of $V_B$ correction would lead to clinically significant quantification errors.

Chapter 5 presents the results for pharmacokinetic modeling with another nitroimidazole based hypoxia tracer: $^{18}$FHX4. Eight patients diagnosed with NSCLC received a dynamic PET scan spanning 4.5 h (including two “coffee breaks”), providing the opportunity to study kinetics up to late time points. Consistent with the results obtained for $^{18}$FFAZA in chapter 3, $^{18}$FHX4 kinetics were found to be reversible ($2T4k+V_B$) and simplified methods performed well, particularly TBr ($R^2 = 0.96$ when compared to NLR derived $V_T$). Modeling results for the complete 4.5 h dataset were found to be equivalent to those for the first 2.5 h, leading us to conclude that imaging at 2.5 h p.i. could suffice for quantification of $^{18}$FHX4 uptake. It would be interesting to see if this conclusion holds at the voxel level, for instance when analyzing the data using spectral analysis.

On the development of hypoxia tracers

The observation that $^{18}$FMISO kinetics are slow, leading to limited image contrast within a clinically feasible time interval between injection and imaging,
has sprung the development of myriad alternative hypoxia tracers\textsuperscript{1}. Most of these (like $^{[18]F}$FMISO, $^{[18]F}$FAZA and $^{[18]F}$HX4) are nitroimidazole based and should therefore, theoretically, have the same uptake mechanisms in tissue. Differences lie in the partition coefficient of the tracer molecules. A hypoxia tracer with a relatively high partition coefficient ("lipophilic") will likely more easily diffuse across the cell membrane, but the relatively low hydrophilicity may prevent such a tracer from diffusing to distant hypoxic cells. This would pose a problem for imaging "diffusion limited hypoxia" as these hypoxic cells will be located a fair distance from the capillaries. In contrast, a hydrophilic tracer will more easily reach remote tissue areas, but diffusion into the cell could be limited or even be prevented by the (lipid-rich) cell membrane.

A lot of effort is invested in finding a tracer with the ideal balance between these properties. This competition between multiple tracers that are really not that different seems an inefficient use of resources. To illustrate, Figure 7.3 demonstrates the similarities between $^{[18]F}$HX4 and $^{[18]F}$FAZA kinetics. The figure shows averaged VOI\textsubscript{Tumor,viable} TAC ($\pm$ 2 SD) from the eight NSCLC patients from the study presented in chapter 5 and seven NSCLC patients imaged with $^{[18]F}$FAZA in a trial currently ongoing at VU University medical center. Imaging protocol of the latter was as described in chapter 3, but augmented with 30 min PET scans at 2 h p.i. Coregistration and VOI\textsubscript{Tumor,viable} TAC generation was performed as described chapter 5. In the figure, TAC were normalized to injected dose and body surface area to allow for comparison.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6_1.png}
\caption{Averaged TAC for eight patients imaged with $^{[18]F}$HX4 (white squares) and seven patients imaged with $^{[18]F}$FAZA (grey squares). Shown are SUV\textsubscript{BSA} as well as TBr curves. For clarity, error bars ($\pm$ 2 SD) are only shown for time points $> 0.1$ h (upwards for $^{[18]F}$FAZA, downwards for $^{[18]F}$HX4).
\label{fig:6.1}}\end{figure}

It seems contradictory that for $^{[18]F}$FAZA and $^{[18]F}$HX4 kinetics were found to

\textsuperscript{1} An overview of clinically evaluated hypoxia tracers is given in Table 2.1. Many other hypoxia tracers are currently in preclinical stages.
be reversible, while for $^{18}$F FMISO often an irreversible model is used based on the uptake mechanism of nitroimidazole \cite{111, 213, 214, 216}. This may be an example of differences in modeling approaches: theory-driven or data-driven. Both methods have advantages as well as disadvantages. In theory-driven methods the model is based on theoretical hypotheses, often corroborated by in vitro data. In data-driven methods the model yielding the best fits to acquired data is selected from a set of standard models. The latter method can therefore be quite sensitive to the quality of the data, e.g. signal-to-noise ratio, patient motion, the bolus injection etc. A theory-driven model will be less sensitive, but the hypotheses on which it is based need to be correct to prevent quantification errors. It may be that for $^{18}$F FMISO the reversible component ($k_4$) is indeed absent, as is assumed in the theory-driven models. In that case it would be interesting to investigate why $^{18}$F FAZA and $^{18}$F HX4 models were found to be different.

This raises questions on whether these tracers are perhaps too hydrophilic for the tracer to diffuse across the cell membrane. However, preclinical results do indicate retention of $^{18}$F FAZA in hypoxic cells \cite{182}. Furthermore, Shi et al. confirmed a negative correlation between perfusion and $^{18}$F FAZA uptake as assessed with $2T4k+V_B$ \cite{137}, which also indicates retention.

Another possible cause could be lack of hypoxic tissue in the tumors that were analyzed in chapters 3, 4 and 5. Indeed this was not verified in the studies. However, given the size of the tumors, the presence of necrotic tissue and the observed increase of TBr over time, it seems unlikely that hypoxia was not present in the tumor tissue evaluated. Furthermore, unpublished data from ongoing analysis, comparing $^{15}$O H$_2$O $K_1$ to $^{18}$F FAZA $V_T$ values for the dataset used in chapters 3 and 4, do show presence of clusters of voxels where perfusion is low while $^{18}$F FAZA uptake is high (unpublished, preliminary results), indicating $^{18}$F FAZA accumulation in low-perfused regions.

A more plausible explanation is that it is an effect of the heterogeneous distribution of hypoxia within the tumor. TAC derived from PET will always be averaged over some tissue volume. With heterogeneity in tracer uptake, this averaging could result in the TAC to appear to show reversible kinetics, i.e. the averaging can lead to a “virtual $k_4$” \cite{216}. Even though in the model selection studies presented in chapters 3 and 5, multiple VOI were considered and no marked differences in kinetics were found, heterogeneity at the intra-voxel level could theoretically still have caused a virtual $k_4$. This would, however, not explain why $^{18}$F FMISO does not show the same.

Finally, it may be that $^{18}$F FMISO kinetics are in fact also reversible, but that $k_4$ is so small that the reversibility is not yet apparent over the time interval of imaging (limited not only for logistic reasons but also by the limited half-life of $^{18}$F (109 min).

\footnote{Interestingly Bruehlmeyer et al. do identify a \textit{reversible} model for $^{18}$F FMISO in glioblastoma multiforme \cite{85}}
On clinical applications
As mentioned in chapter 2 hypoxia PET imaging would have several benefits in a clinical setting. Apart from determining prognosis and monitoring therapy response, hypoxia imaging could be used to enhance treatment efficacy and to prevent unnecessary side effects in individual patients by aiding in personalized selection of an appropriate treatment strategy.

1. Radiation dose manipulation
Recent advances in radiation therapy technique enable manipulation of administered dose levels with high precision. Along with current developments on respiratory gating on the diagnostic as well as on the therapeutic side, radiation dose painting based on hypoxia PET images is possible from a technical standpoint [166, 197, 217, 218, 219, 220, 221, 222]. Trials investigating the efficacy of localized dose level boosting are currently recruiting patients (NCT01576796, NCT01024829).

Boost trials based on hypoxia imaging [223, 224, 225, 226] are inherently complex: dose boosting of specified tumor areas will increase overall dose to the tumor which could in itself lead to increased efficacy (as well as to increased adverse effects). On the other hand, boosting whilst maintaining overall dose level implies dose levels be reduced in areas not identified by PET as hypoxic. Due to perfusion limitations in combination with partial volume effects, hypoxia PET imaging may miss highly hypoxic areas [227].

2. Treatment selection
An alternative approach is to use PET hypoxia imaging for personalized therapy selection, for example for treatment with radiosensitizers [80] or hypoxia-specific chemotherapy [73, 74, 75]. As these pharmaceuticals are associated with increased adverse effects, careful selection of patients who would benefit from these type of treatments is required. PET hypoxia imaging could be used to classify tumor tissue as “nonhypoxic” or “hypoxic”.

The imaging time point can strongly influence hypoxic volume delineation. For example, at early time points, hypoxia tracer concentrations are expected to be high in areas of high perfusion and low in areas of low perfusion. Over time, due to clearance from normoxic cells and accumulation in hypoxic cells, the relative difference between tracer concentrations in these areas will likely change. However, in an absolute sense, areas of high perfusion may still contain higher activity concentrations than hypoxic areas, even up to late time points.

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3 For example, high PET signal pertaining from highly hypoxic cells could be averaged out by lack of signal in adjacent necrotic areas.
Therefore, (static) imaging should be performed at late time points, when the balance between activity concentrations in hypoxic areas and normoxic areas is such that contrast between hypoxic and normoxic areas is sufficient for delineation. However, identifying a single optimal time point for imaging of all areas within a tumor is nontrivial: the rate of hypoxia tracer accumulation will depend on the amount of hypoxic cells and levels of hypoxia, which will vary throughout tumor tissue. In case of reversible specific uptake (as was indicated for $^{18}$FFAZA and $^{18}$FHX4), tracer clearance from hypoxic tissues will complicate defining the optimal imaging time point further. In contrast to simplified quantification methods, parametric methods take into account the shape of the TAC and are therefore more capable of accounting for local differences in kinetics. It would be interesting to see whether parametric results are indeed more consistent with direct measures of tissue pO$_2$ tension$^4$ than has previously been found for simplified methods [158, 228, 229].

In dose painting, quantification and delineation errors could cause highly hypoxic cells to receive a reduced radiation dose. Therefore, parametric methods would be recommended to quantify tracer uptake at the voxel level (particularly spectral analysis as this method also corrects for $V_B$). For treatment selection purposes, perhaps a less precise quantification method would be sufficient. In that case, static PET hypoxia imaging in combination with a validated simplified parameter might be an option.

Before commencing clinical trials with hypoxia-imaging based dose painting strategies, the link between perfusion and hypoxia imaging should be investigated further. Given the hypothesized link between hypoxia and perfusion combined PET imaging with hypoxia and perfusion tracers may be required for accurate delineation of hypoxic regions. Unfortunately, perfusion PET imaging using $^{15}$O$\text{H}_2\text{O}$ requires presence of an onsite cyclotron. Strategies have been proposed for estimating the level of perfusion from a dynamic hypoxia PET scan [112]. However, although hypoxia tracers are designed to readily diffuse through tissue, diffusion rates will not be as high as for $^{15}$O$\text{H}_2\text{O}$. Furthermore, $^{15}$O$\text{H}_2\text{O}$ is metabolically inert while hypoxia tracers are designed to react with molecules present within cells. Although this implies that hypoxia tracer K$_1$ values may not be an accurate measure of perfusion, early hypoxia tracer uptake may be indicative of perfusion levels (as demonstrated by Bruehlmeier by comparing $^{18}$FFMISO uptake with $^{15}$O$\text{H}_2\text{O}$ in patients with glioblastoma multiform [85]).

$^4$ As found by Bartlett et al. for $^{18}$FFMISO in a preclinical setting [212]
Outlook

For clinical applications, a standardized method for accurate translation of hypoxia PET images into clinical decisions is required. Ideally, the optimal hypoxia tracer, the optimal yet clinically feasible imaging protocol, a validated method for quantification of hypoxia and clinically relevant threshold values for classification or delineation, should be determined.

Many hypoxia tracers are currently under evaluation and comparing tracer performance has proven difficult. To date, mixed results have been found in studies comparing hypoxia tracer uptake to “gold standard” methods such as polarographic electrode measurements and pimonidazole staining. This stresses the need for validated methods for quantification. Only when using validated quantification methods, can definite conclusions be drawn on the performance of specific hypoxia tracers and can different tracers be compared.

Translating hypoxia PET images into clinical decisions involves:

1. Translating PET images to “specific uptake” of the hypoxia tracer.
2. Translating “specific uptake” to tissue pO\textsubscript{2} levels and verifying hypoxia-specificity.
3. Translating tumor pO\textsubscript{2} levels to clinically relevant hypoxia levels.
4. Translating results on clinically relevant hypoxia levels into clinical decisions.

Figure 7.4 provides an overview of research steps to be performed subsequently when developing a validated method for quantification of hypoxia. This is by no means a complete list of all research that should be performed before a novel PET tracer is introduced in a routine clinical setting, but it does provide an overview of some important steps to ensure correct interpretation of hypoxia PET images and to avoid having to repeat clinical trials.

In developing a hypoxia PET imaging biomarker, first, the hypoxia-specificity of the tracer’s uptake should be verified. This can be achieved by inducing hypoxia in (tumor) cells and comparing tracer uptake. As inducing hypoxia in patients would be unethical, this step is performed in a preclinical setting (\textit{in vitro} and \textit{in vivo}). Preclinical assessment often includes other proof-of-concept studies such as comparison of tracer distribution to “gold standard” methods for assessing tissue pO\textsubscript{2} levels, and pharmacokinetic modeling. However, preclinical results cannot necessarily be directly translated to the clinical setting. Therefore, ideally, all findings should be verified in patients. Due to ethical considerations, patient studies are inherently less controlled and careful selection of trial participants is key. Pharmacokinetic modeling can be used to translate
Figure 6.2: Overview of some important translational research steps to develop valid PET imaging biomarker for hypoxia.

1 Translating PET images to “specific uptake”
2 Translating “specific uptake” to tissue pO₂ levels and verify hypoxia-specificity.
3 Translating tumor pO₂ levels to clinically relevant hypoxia levels.
4 Translating results on clinically relevant hypoxia levels into clinical decisions.

PET images into information on specific uptake and provide insight into other factors contributing to the PET signal. As pharmacokinetic modeling requires an elaborate imaging protocol and extensive analysis, the aim is to find simplified methods (such as SUV) that provide an acceptable estimate of specific uptake. Even if a suitable parameter can be identified, the parameter will not yet be validated for use in clinical value assessment. First, consistency of tracer specific uptake and the influence of perfusion and heterogeneity should be investigated. If found necessary, the quantification method should then be adjusted accordingly.

Given the complexity of interpreting hypoxia PET images, hypoxia imaging alone may not be enough for accurate quantification. Multiparametric imaging could lead to a better understanding of how to interpret hypoxia PET images and could an important step towards translating hypoxia PET images into clin-

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It should be noted that even though pharmacokinetic modeling is more capable of separating between compartments than simplified methods, the method may still be unable to truly extract a measure for “specific” uptake, i.e. k₃ or BP (as was observed for [¹⁸F]FCH due to inability to distinguish between K₁ and k₃ and for [¹⁸F]FAZA and [¹⁸F]HX4 due to data noise levels).
ical decisions. At the least, consecutive imaging with tracers for perfusion and hypoxia would be recommended\(^6\). Additionally, a combination with \(^{18}\text{F}\)FDG PET imaging to assess tissue viability may be useful since the nitroreductase enzymes required for the presumed hypoxia-specific uptake mechanism can only be present in viable cells. High hypoxia PET signal in areas of limited \(^{18}\text{F}\)FDG uptake may then indicate presence of a limited concentration of viable yet highly hypoxic cells\(^7\). This method may thereby help to account for partial volume issues arising from the heterogeneous distribution of hypoxia in tissue\(^8\).

Ideally, biological validation would be performed on the technically validated parameter to verify the hypoxia-specific uptake mechanism, as assessed preclinically, in patient studies and to translate parameter values to tissue pO\(_2\) levels. However, “gold standard” methods for assessing pO\(_2\) levels are highly invasive and it remains unclear which level of pO\(_2\) tension actually designates clinically relevant hypoxia. Moreover, levels are thought to differ between tissue (and tumor) types. Alternatively, biological validation could be performed in a limited amount of clinical studies only to verify the hypoxia-specificity and research could move on to translating parameter values into clinically meaningful measures directly.

Once the quantification method has been validated, and only then, can the tracer be used in clinical trials investigating the clinical value of the method. Prognostic and predictive significance of tracer uptake and possibly associated threshold values for classification could then be derived. To enable assessment of the performance in a response monitoring setting, further validation would be required since, apart from response, the treatment itself may well influence tracer uptake (such as changes in perfusion and tracer plasma metabolism). Finally, the efficacy of hypoxia-targeted therapies can only be evaluated when patients participating in the trial have been carefully selected based on the presence of hypoxic tumor tissue (as determined by a validated quantification method). The performance of hypoxia dose painting strategies can only be assessed when a validated quantification method is used to determine appropriate local dose levels.

The considerations discussed in this chapter summarize some of the challenges that need to be overcome before hypoxia PET imaging can be implemented in a routine clinical setting. With continuing technological and methodological

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\(^6\) As perfusion assessment would in this case be used only for interpreting the hypoxia PET signal, perhaps a semiquantitative method would suffice and perfusion assessment could be performed using techniques more feasible in a routine clinical setting, such as CT or MRI, rather than using dynamic \(^{15}\text{O}\)H\(_2\)O PET imaging.

\(^7\) Alternatively, such a discrepancy could also be caused by presence of hypoxia tracer in interstitial fluids in these areas. Therefore, results should be interpreted with care.

\(^8\) It should be noted that due to the long imaging time intervals required for hypoxia imaging and the relatively long half-life of \(^{18}\text{F}\), such a combined imaging protocol would require imaging on separate days. Consequently, coregistration issues are to be expected.
advances, clinical applicability of hypoxia PET imaging is improving. However, successful clinical implementation for personalized therapies can only be achieved through rigorous validation studies.