Review Article
Kinetic modeling in PET imaging of hypoxia

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Abstract: Tumor hypoxia is associated with increased therapeutic resistance leading to poor treatment outcome. Therefore the ability to detect and quantify intratumoral oxygenation could play an important role in future individual personalized treatment strategies. Positron Emission Tomography (PET) can be used for non-invasive mapping of tissue oxygenation *in vivo* and several hypoxia specific PET tracers have been developed. Evaluation of PET data in the clinic is commonly based on visual assessment together with semiquantitative measurements e.g. standard uptake value (SUV). However, dynamic PET contains additional valuable information on the temporal changes in tracer distribution. Kinetic modeling can be used to extract relevant pharmacokinetic parameters of tracer behavior *in vivo* that reflects relevant physiological processes. In this paper, we review the potential contribution of kinetic analysis for PET imaging of hypoxia.

Introduction

The majority of locally advanced solid tumors exhibit hypoxic and even anoxic regions that are heterogeneously distributed within the tumor mass [1]. These regions are a result of an imbalance between oxygen supply and consumption and can generally be divided into perfusion limited (acute) hypoxia, which is generated by the diffuse nature of tumor vasculature, and diffusion limited (chronic) hypoxia, which develops as the distance between tumor vasculature, and the expanding tumor cells increases. Additionally, cancer associated anemia may contribute to the formation of tumor hypoxia following a decrease in the bloods ability to carry the oxygen [2].

The intricate link between tumor hypoxia and increased malignancy is well established [3-5]. In addition, hypoxic tumor cells represent an important therapeutic problem because of increased resistance towards ionizing radiation and chemotherapy [6-9]. The therapeutic targeting of tumor hypoxia represents an attractive strategy for individualized therapy of cancer patients. In radiation oncology different approaches such as the use of adjuvant hypoxia sensitizers (e.g. nimorazole) during radiation therapy [10, 11]; therapeutic combinations aiming to increase tumor oxygenation [12-14]; and modern high linear energy transfer radiation strategies (e.g. heavy ion therapy) [15] has been applied with varying results. Additionally, heterogeneous delivery of radiation, also termed dose painting, holds the potential to selectively increase radiation dose to areas with known therapeutic resistance, such as hypoxic tumor regions. This approach is extremely challenging, as it requires the ability to continuously identify regional changes in intratumoral oxygenation levels, and currently knowledge is very limited with regard to how regional oxygenation fluctuates during therapy [16, 17]. Therefore methods that allow for identification of patients with hypoxic tumors that would benefit from hypoxia-modified treatment could improve treatment efficacy. However, presently no method has reached a position as a clinically accepted routine approach for identification of tumor hypoxia, even though a number of techniques have been evaluated to deter-
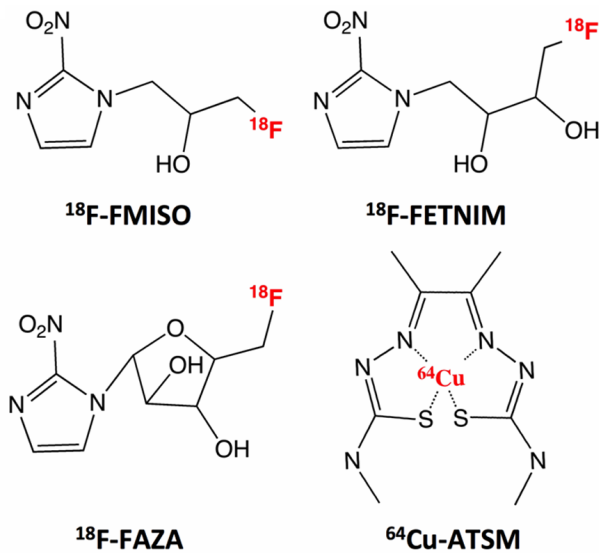
Figure 1. Chemical structures of hypoxia specific PET tracers. ^{18}F-FMISO: ^{18}F-fluoromisonidazole; ^{18}F-FETNIM: ^{18}F-fluoroerythronitroimidazole; ^{18}F-FAZA: ^{18}F-fluoroazomycin; ^{64}Cu-ATSM: [64]Cu(II)diacethyl-bis(N4-methylthiosemicarbazone). Besides ^{64}Cu, Cu-ATSM can also be labeled with other radioactive copper isotopes such as [60]Cu, [61]Cu and [62]Cu.

mine oxygenation in tissue. The polarographic electrode is currently the only method that can provide a direct measure of oxygen tension, and it has been considered as the gold standard for measurement of tumor pO[2] over the last two decades [4, 18-20]. In addition, a different type of electrode based on the principle of oxygen-induced quenching of light emitted by fluorescent dye, has also been used for this purpose [21-23]. Immunohistochemical staining of exogenous or endogenous surrogate markers of hypoxia in tumor biopsies is another approach often used to assess tumor oxygenation [24-26]. However, these needle-based methods have some limitations as they are invasive procedures and only applicable for tumors that are accessible with a needle. Moreover, these techniques only allows for the assessment of oxygenation in a limited volume of the tumor microenvironment. The heterogeneous distribution of hypoxic areas makes a non-invasive imaging approach attractive, and different techniques such as magnetic resonance imaging (MRI) and electron paramagnetic resonance (EPR) with oxygen sensitive probes have been applied for assessment of tumor oxygenation [27-30]. Additionally, positron emission tomography (PET) offers *in vivo* measurement and quantification of physiologi-

cal processes with high temporal and adequate spatial resolution.

At present the radiolabelled glucose analog, 2-deoxy-2-(^{18}F)fluoro-D-glucose (^{18}F-FDG) is the most used PET tracer in clinical oncology. It utilizes that cancer cells take up greatly elevated levels of glucose, known as the Warburg effect [31, 32]. Additionally, ^{18}F-FDG has also been proposed as a surrogate marker of tumor hypoxia following the potential increased cell metabolism from oxidative phosphorylation to glycolysis when oxygen level drops [33]. This induce an increase in the uptake of glucose but despite this well-characterized connection, preclinical and clinical studies have reported conflicting results; but in general ^{18}F-FDG cannot be considered as a consistent surrogate marker of hypoxia in tumors [34-39]. Accordingly, several radiotracers for specific PET imaging of hypoxia including ^{18}F-Fluoromisonidazole ^{18}F-FMISO, fluoroazomycin (^{18}F-FAZA), fluoroerythronitroimidazole (^{18}F-FETNIM) and copper(II)diacethyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) have been developed (**Figure 1**).

One of the advantages of PET is the ability to measure radiotracer concentration in tissue or organs. Semiquantitative approaches are often used for analysis of PET images but the main drawback is that it does not take into account variations caused by underlying processes such as tracer delivery, trapping, competition with other molecules, and physical clearance [40]. Dynamic PET can be used to study tracer pharmacokinetics and temporal changes in uptake, and clinical studies have indicated that valuable additional information can be obtained from the profile of time activity curves (TACs) [32, 41, 42]. Furthermore, mathematical modeling based on non-invasive imaging data can possibly be used to extract meaningful parameters of tracer accumulation and distribution kinetics [40, 43-46].

Basically, tracer kinetic modeling is based on a compartmental model that is comprised of a number of functional, homogenous units termed compartments. These are interpreted as separate, structureless pools of tracer in distinct state. The tracer transport between compartments can be described by rate param-
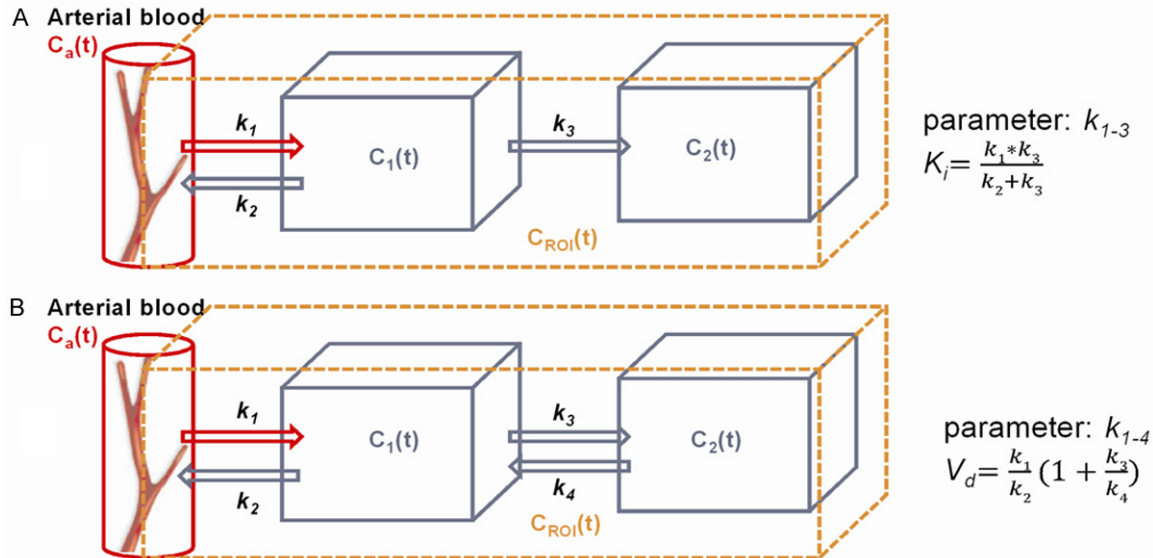
Figure 2. Graphical representation of common 2-tissue compartment models. Model (A) is consisting of three components and each is a function of time expressed as time activity curves. Input function, $C_a(t)$ consists primarily of arterial blood and interstitial space close to vessels. The $C_1(t)$ compartment represents unbound tracer in tissue whereas $C_2(t)$ compartment represents bound tracer. This model contains three kinetic parameters, k_{1-3}. Model (B) contains the same number of compartments but kinetic parameter also includes the transport rate constant k_4. Moreover, the netto influx parameter, K_i can be calculated based on k_{1-3} in a irreversible model, and k_{1-4} can be used to calculate the distribution volume, V_d in a reversible model.

eters, and usually the models are described mathematically by first order differential equations. **Figure 2** demonstrates the two most widely used model structures within tracer kinetic studies. While a 2-tissue reversible model is often applied in studies of neuroreceptor-ligands, a 2-tissue irreversible model has been implemented in most studies of hypoxia tracer kinetics [47, 48]. In principle, additional compartments can be added to a model in order to obtain a more realistic interpretation of tracer kinetic behavior *in vivo,* including an increasing number of parameters. However, increased complexity affects the accuracy and reliability of the model parameter estimation due to limitation of the nonlinear minimization problem [49]. Therefore model selection should be considered as a balance between statistical accuracy and model complexity. As an alternative, graphical analysis is a more computational efficient way to calculate combinatorial parameters by turning a nonlinear problem into linear plots [50]. Thus the slope of the linear part of Patlak and Logan plots can be used to determine netto influx rate, K_i and distribution volume, V_d for tracers with irreversible and reversible uptake, respectively.

Overall compartmental analysis provides a possibility to understand the tracer behavior in a specific tissue and allows for the derivation of important kinetic parameters. This can provide additional information on a metabolic processes at the molecular level and thereby potentially improve the diagnostic and prognostic potential of a PET tracer. This review focuses on studies where compartment/kinetic modeling has been applied on PET data for quantification of hypoxia.

^{18}F-FMISO

The majority of hypoxia PET tracers belongs to a group of compounds termed nitroimidazoles that have been used intensively as immunological markers for immunohistochemical procedures and flowcytometry [51-55]. Nitroimidazoles enter cells by diffusion and are reduced by nitroreductases inversely correlated with oxygen tension. In the presence of oxygen they are able to leave the cell again, but under hypoxic conditions the nitroimidazoles become reduced and will be irreversibly trapped within the cells [56, 57].

[18]F-Fluoromisonidazole (^{18}F-FMISO) was the first nitroimidazole-based radiotracers for
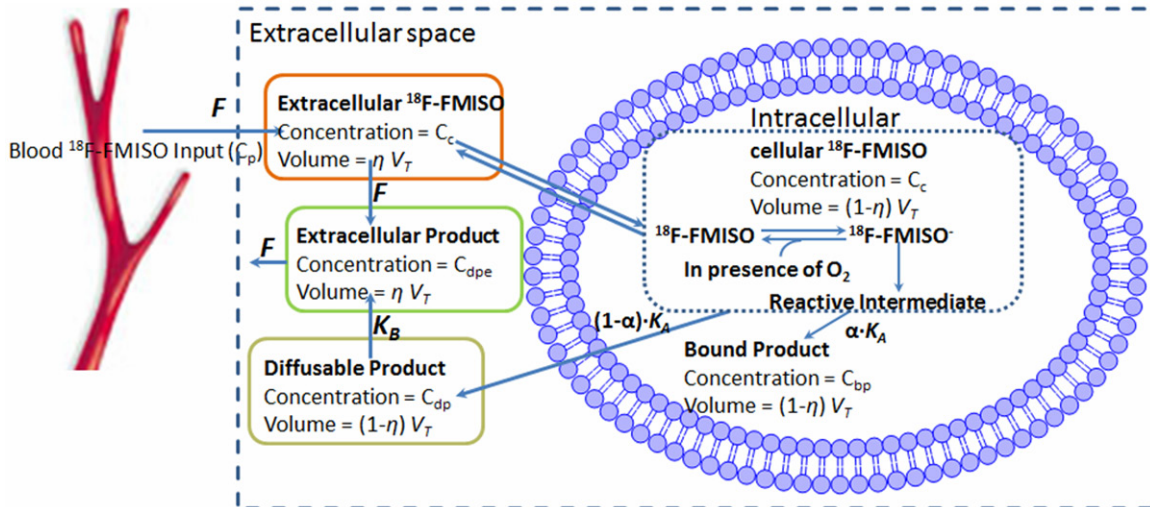
Figure 3. The model of ^{18}F-FMISO transport and metabolism presented by Casciari *et al*. ^{18}F-FMISO enters the target tissue by flood flow (*F*) and diffuse across the cell membrane, where it is reduced. K_A is the rate constant describing the rate by which FMISO is reduced. Cellular accumulation of FMISO and washout of diffusible product is running side by side. C_c, C_p, C_{bp}, C_{dp}, C_{dpe} corresponds to ^{18}F-FMISO concentration in tissue, in blood plasma, ^{18}F-FMISO bound product and cellular diffusible product, respectively. V_T, η are the tissue specific volume and dimensionless fractional variable. K_A is related to the oxygen level and can be considered as a surrogate measure of hypoxia.

hypoxia PET imaging to be developed, and it has been extensively used in both preclinical and clinical studies [58-63].

The kinetic profile and characteristics of ^{18}F-FMISO has also been investigated in a number of studies applying various approaches. In 1995 Casciari *et al*. developed a mathematical model based on knowledge on the cellular metabolism and tracer transport in tissue. The main objective was to quantify ^{18}F-FMISO reaction rate constant, K_A, a parameter related to the level of cellular oxygen (see **Figure 3**). Models performance was tested using Monte Carlo simulation and fitted to ^{18}F-FMISO time-activity PET data from human and rat tumors. This demonstrated the importance of including some transport limits such as parameter fixing of the compartments representing tracer in the tissue. In addition the effect of noise on accurately determination of K_A was small when some parameters were fixed at physiological meaningful values; however, the accuracy of K_A was sensitive to the accuracy of the fixed parameters [64].

Kelly and Brady also did a simulation work on the spatiotemporal distribution of ^{18}F-FMISO in tumor microenvironment by applying a modular approach to simulated data. They included parameters to model spatial diffusion of free

^{18}F-FMISO and its reduced compounds and modified a conversional reversible 2-compartmental model, with a reaction-diffusion equation, in order to improve ^{18}F-FMISO distribution dynamics [65]. The model was then used to generate simulated data based on patient plasma time activity curve as input. It was necessary to set transport parameter k_4 = 0. Additionally, a number of technical limitations that influenced the model were identified. On the basis on this, the kinetic and spatial effects of diffusion could be disregarded in the data fitting process. The adaptive model illustrated that a reversible 2-compartment model with varying diffusion distances and diffusion coefficients was able to generate more realistic TACs. Moreover, it demonstrated that simulation models of tracer spatiotemporal distributions provides a options to investigate the effects of heterogeneity on TACs and the relationships between image data and molecular processes, prior to empirical studies.

Besides these simulation studies of ^{18}F-FMISO, a limited number of preclinical studies in mice and rats have focused on kinetic modeling. Whisenant *et al*. did a study in nude mice bearing Trastuzumab-resistant breast tumors in order to test the reproducibility of kinetic parameters derived from dynamic PET scan protocols [66]. Mice received 60 min dynamic

PET scan six hours apart with ^{18}F-FLT and ^{18}F-FMISO, respectively. They found that the distribution volume, V_d and the net influx constant, K_i were the most reproducible parameters for ^{18}F-FMISO. In contrast transport constant k_1 and trapping constant k_3 were the most variable. For ^{18}F-FLT V_d, k_3, and K_i were shown to be the most reproducible parameters.

In another study Bejot *et al.* evaluated ^{18}F-3-NTR, a 3-nitro-1,2,4-triazole analogue (N(1) substituted) (^{18}F-3-NTR) against ^{18}F-FMISO as hypoxia PET tracer in tumor bearing mice and concluded that ^{18}F-3-NTR could not be considered a hypoxia imaging agent due to its poor binding capacity [67]. Furthermore, based on the Akaiki information criterion there was no benefit of applying a 3-compartment irreversible model to describe ^{18}F-FMISO kinetics when compared to a 2-compartment irreversible model.

In a recent study, Bartlett *et al.* investigated a group of nude rats bearing prostate tumors in order to find a non-invasive approach based on dynamic PET for better identification of tumor hypoxia [23]. Voxelwise kinetic parameters calculated from 2-compartment model fitting to ^{18}F-FMISO uptake data were compared to pO_2 measurements. When k_1 and k_2 were constrained, ^{18}F-FMISO trapping rate, k_3 was shown to be a more robust discriminator for low pO_2 within tumor tissue than a simple tissue-to-plasma ratio. This suggest that voxelwise based kinetic modeling could improve accuracy of tumor hypoxia estimation.

Finally, the use of kinetic modeling of ^{18}F-FMISO has also been investigated in a few clinical studies. Bruehlmeier *et al.* used ^{15}O-H$_2$O to study the influence of perfusion on the pharmacokinetics of ^{18}F-FMISO in eleven patients with brain cancer and observed an increased late uptake in glioblastomas [68]. Pixel-wise comparison showed a positive relationship between ^{15}O-H$_2$O and early uptake of ^{18}F-FMISO, whereas no correlation was found for late ^{18}F-FMISO distribution. Standard 2- and 3-compartment models and Logan graphical analysis were used to calculate distribution volumes and transport rate constants and found that a V_d above one was indicative of active ^{18}F-FMISO uptake. Additionally, the ^{18}F-FMISO uptake rate, k_1 was increased in all tumor tissue, compared to values in white matter and in cortex. In

meningioma they found the highest value for k_1 of all brain tumors. This increase k_1 permitted delineation of the meningioma in an early ^{18}F-FMISO PET image, but the tumor did not exhibit subsequent ^{18}F-FMISO accumulation and was not visualized in the late PET images. Accordingly, the ^{18}F-FMISO distribution volume in the meningioma was not increased despite a high k_1 value. However, despite V_d providing a good measure of ^{18}F-FMISO accumulation, it was concluded that it does not offer additional information compared to tumor-to-background ratios of late PET images.

Using a similar experimental setup with ^{15}O-H$_2$O and ^{18}F-FMISO, Bruehlmeier *et al.* applied graphical analysis on dynamic PET data from six dogs with spontaneous sarcomas [69]. They found that tumor tissue could be markedly delineated from surrounding tissue including muscles by positive influx rate K_i derived from a Patlak plot. The presence of tumor hypoxia was confirmed by eppendorf electrode measurement.

In a study of head and neck cancer patients, Thorwarth *et al.* performed quantitative image analysis based on irreversible compartmental modeling of dynamic ^{18}F-FMISO PET data [70]. This model was different from previous approaches by including weighing factors for the respective model compartments. The input function was determined from the signal of a reference tissue and the irreversible model was applied to identify and quantify TACs using a voxel-to-voxel approach. Evaluation of model performance indicated that the parameters derived from the kinetic model were superior to SUV for quantification in extremely low oxygenated and necrotic tissue areas. In another study the same model was applied to a small group of head and neck cancer patients that were scanned dynamically for 60 min prior to radiotherapy [42]. Assessment parameters representing hypoxia and perfusion derived from the kinetic model were not able to predict treatment outcome. However, Thorwarth *et al.* introduced a novel parameter, termed the malignancy value, that was dependent on both hypoxia and perfusion characteristics of the tissue. This malignancy value could be used as a prognostic factor indicating that the parameters may provide additive information. Furthermore, in a preclinical study, Cho *et al.* adapted Thorwarth's model for comparing perfusion

and hypoxia parameters derived from MRI with ^{18}F-FMISO PET in a rat prostate cancer model [71]. The tumor perfusion derived from DCE-MRI was found positively correlated with early ^{18}F-FMISO PET but inversely correlated to late slope maps of the ^{18}F-FMISO PET time-activity curve.

Wang *et al.* applied a generic irreversible 2-compartmental model to analyze dynamic ^{18}F-FMISO PET dataset within three regions of an image phantom [72]. The purpose of the phantom study was to determine the statistical accuracy and precision of the kinetic analysis. The results from the phantom study was used for guidance in a clinical dynamic ^{18}F-FMISO PET study of nine head and neck cancer patients with local squamous cell carcinoma [73]. Based on this they identified K_i as a potential hypoxia marker and found a significant correlation to ^{18}F-FMISO tumor-to-blood ratio.

In addition to modeling of tumor hypoxia, ^{18}F-FMISO has also been used in studies of ischemic stroke. Takasawa *et al.* performed a rat stoke study that included dynamic PET with ^{18}F-FMISO. In order to obtain quantitative comparison between the tracer retention from affected and unaffected cerebral hemispheres, an irreversible compartmental model was used for calculation of k_1 and K_i [74]. Immediately after the occlusion of the middle cerebral artery a remarkable increased K_i was observed in the affected site. This suggests that ^{18}F-FMISO can potentially be used to visualize the occlusion at a very early stage. However, in spite of histological findings, no difference in either k_1 and K_i was found between affected and unaffected cerebral hemispheres 48 hours after the occlusion. In another study Hong *et al.* used the dataset generated by Takasawa *et al.* to perform kinetic analysis [75]. Basis functions from plasma input compartment (BAFPIC) model is a modified version of the standard compartment model approach and can be applied to both reversible and irreversible models. In this study BAFPIC was compared to both conventional compartment modeling, as well as the most common analysis methods for generation of parametric maps. The BAFPIC method showed lower variability and bias than nonlinear least squares modeling in hypoxic tissue. In addition, voxel based parametric mapping of dynamic imaging was shown to be less affected by noise-induced variability compared to nonlin-ear least squares modeling and Patlak graphical analysis. BAFPIC could therefore potentially be applied to other tracers with irreversible characteristics, and although the focus of this study was on voxelwise modeling, other kinetic parameter estimations in regions with noise can be performed using the BAFPIC algorithm as well.

^{18}F-FETNIM

Even though ^{18}F-FMISO has been used extensively, a slow hypoxia specific retention and clearance from non-hypoxic tissue is a limitation and results in low tumor-to-background contrast [76]. To improve image quality ^{18}F-labeled nitroimidazole analogs including ^{18}F-FETNIM, 2-nitroimidazole nucleoside analog (^{18}F-HX4) and 2-(2-Nitromidazole-1H-yl)-N-(2,2,3,3,3-pentafluoropropyl)acetamind (^{18}F-EF5), that have structural modifications - with changed lipophilic properties, have been developed to overcome the basic pharmacokinetic limitations [44, 77, 78]. ^{18}F-FETNIM was introduced as a novel hypoxia-specific PET tracer in 1995 and has shown promising results in clinical studies [77, 79].

In studies in patients with head and neck cancer Lehtiö *et al.* performed compartmental analysis based on dynamic ^{18}F-FETNIM PET scans [80, 81]. They adapted the model previously introduced by Casciari *et al.* for ^{18}F-FMISO metabolism and transport (**Figure 3**). Blood flow rate (F), tissue activity correction factors ($\beta1$, $\beta2$) and cellular ^{18}F-FETNIM reaction rate (K_A), considered the hypoxia specific binding rate, were estimated by fitting the model to dynamic PET data. They found that the level of tracer uptake (K_A) was most sensitive to changes in oxygen in tissue with high blood flow. Furthermore, distribution volumes derived from Logan plots correlated positively with the tumor-to-plasma ratio but not with tumor-to-muscle ratio. This suggests that plasma should be preferred as reference tissue rather than muscle.

^{18}F-FAZA

Like ^{18}F-FETNIM, ^{18}F-FAZA is a next generation nitroimidazole-based PET tracer. Several studies have compared the tracer kinetics of ^{18}F-FAZA with ^{18}F-FMISO and reported of improvements in washout from non-hypoxic tis-

sue and faster renal clearance [82-84]. Moreover, a recent study of 50 patients confirmed the feasibility of using FAZA PET for detection of tumor hypoxia in clinic [85].

A few studies have focused on kinetic modeling of dynamic ^{18}F-FAZA PET. Reischl et al. demonstrated an increased tumor to blood ratio due to faster vascular clearance of ^{18}F-FAZA when compared to ^{18}F-FMISO and that is one of most important criteria for hypoxia tracer development in relation to hypoxia imaging optimization [86].

Busk et al. reported a dynamic ^{18}F-FAZA animal study in three tumor xenograft models of squamous cell carcinomas of the cervix. The dynamic data were analyzed by an irreversible 2-compartmental model. Overall variations in the pattern of TACs were tumor type dependent. The influx rate constant K_i was shown to have a strong correlation to late ^{18}F-FAZA uptake in two of three tumors models. On the other hand, weak correlation between irreversible parameter k_3 and late ^{18}F-FAZA uptake was observed in two of three tumor models [87].

Two small clinical studies have used dynamic ^{18}F-FAZA PET data to compare different kinetic models. While Shi et al. used a voxelvise approach to correlate model parameters of interest with perfusion measured by ^{15}O-H$_2$O PET in patients with head and neck cancer; Verwer et al. applied the Akaiki information criterion to evaluate model fitting to TACs obtained from nine non-small cell lung cancer patients [88, 89]. Both studies concluded that a reversible 2-compartment model showed the best correlation and robustness with their expectations and assumptions.

Cu-ATSM

Beside the nitromidazole-based compounds, a copper labeled metallocomplex termed copper(II)diacethyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) has been applied as radiotracer for PET imaging of tumor hypoxia [90]. Image quality is generally superior to the nitroimidazoles, however, despite promising clinical performance [91-93], preclinical data from experimental hypoxia imaging is conflicting with regard to tracer selectivity [94-97]. The mechanism of tracer uptake and retention is still not fully understood but it is believed that Cu(II)-

ATSM is reduced to the unstable [Cu(I)- ATSM]$^-$ complex in both hypoxic and normoxic cells. Under normoxic conditions [Cu(I)-ATSM]$^-$ is reoxidized and thereby able to of leave the cell. The reoxidation of [Cu(I)-ATSM]$^-$ is, however, not expected to occur under hypoxic conditions, and the electrochemically negative molecule becomes irreversibly trapped and dissociates [98, 99].

As for the other hypoxia PET tracers there are only limited data concerning the pharmacokinetics of Cu-ATSM. A simulation work carried out by Holland et al. applied a model based on an in vitro study on cellular uptake and retention of ^{64}Cu-ATSM in EMT6 murine carcinoma cells. The model demonstrated that a decrease in cellular pH may protonate the unstable Cu(I)-ATSM complex and increase the rate of dissociation [100]. The kinetic analysis was consistent with experimental cellular uptake.

Bowen et al. suggested an electrochemical models based on the retention mechanisms for ^{18}F-FMISO and ^{61}Cu-ATSM, respectively [101]. In this work the preclinical data were compared to transformation functions derived from tracer uptake and pO$_2$ measurements. Comparisons between ^{61}Cu-ATSM and ^{18}F-FMISO uptake showed inconsistent results, but this could be due to the different retention mechanisms. However, the results suggested that ^{18}F-FMISO uptake was superior for differentiation of a wide range of pO$_2$ values, but ^{61}Cu-ATSM uptake provided more reliable information on variations at low pO$_2$ range.

In another study Dalah et al. performed a simulation work based on the model adapted from Kelly and Brady [102]. This refined model was used to simulate realistic TACs that were comparable to ^{64}Cu-ATSM patient TACs and showed favorable tumor delineation in form of higher tumor to blood ratio compared to ^{18}F-FMISO.

Kinetic modeling of Cu-ATSM PET has also been applied in a few animal studies. Lewis et al. evaluated Cu-ATSM retention in canine models of hypoxic myocardium [97]. Monoexponential analysis and 2-compartmental model fitting were applied to the TACs in order to determinate washout of Cu-ATSM from regions of the myocardium. Based on the assumption that the relationship between washout and retention is inverse proportional, they reported

of an increase of Cu-ATSM retention in ischemic regions when compared to normal myocardium. Moreover, the data also indicated that Cu-ATSM retention in hypoxic regions of the myocardium was independent of increased perfusion and that there was no retention in necrotic tissue.

Recently, McCall *et al*. performed a preclinical study of ^{64}Cu-ATSM uptake in rats bearing FaDu human head and neck cancer xenografts. Graphical analyses were used to derive the net influx parameter, K_i and distribution volume, V_d. In general, a stronger linear relationship was found for Logan plots but both parameters were significantly higher in tumor tissue when compared to muscle tissue. Additionally, a continuous increasing tumor-to-muscle ratio was observed [103].

Finally, Cu-ATSM has been used in multitracer PET studies where two or three tracers were administered with delayed injections. Following image separation, signal recovery was performed based on differences in tracer kinetics and physical decay. In a phantom study of [62]Cu-ATSM and the perfusion PET tracer, [62]Copper-pyruvaldehyde-bis[N4-methylthiosemicarbazone] ([62]Cu-PTSM), Rust *et al*. demonstrated a great similarity between perfusion and hypoxia parameters obtained by the dual tracer approach, and parameters estimated by single-tracer imaging [104]. The results were later confirmed by Black *et al*. that adapted the phantom study protocol and used it for [62]Cu-ATSM and [62]Cu-PTSM PET imaging in dogs with spontaneous tumors [105]. Rate constants obtained from irreversible 2-compartment modeling was used to evaluate the signal separation of the two PET tracers. However, while k_1 and k_2 could be recovered from the mixed PET signal the recovery of k_3 was more problematic. The same group has also experimented with a triple-tracers setup including ^{18}F-FDG [106]. Altogether, the multitracer approach can potential provide different functional information about physiological processes within a short period of time; thereby decreasing the possible effect of microenvironmental changes. However, there are some questions there needs to be address with regard to the long dynamic acquisition time, and the risk of losing information as a consequence of temporal overlap.

Table 1 summaries studies on kinetic modeling of hypoxia using PET tracers.

Challenges in kinetic modeling of hypoxia PET tracers

Studies on kinetic modeling of hypoxia PET tracer needs also deal with the issues, related to acquisition of dynamic PET data in general. The amplitude of noise can be influenced by several physical factors such as the radiopharmaceutical properties of the tracer, injected dose, frame duration, and the sensitivity of the PET camera. In addition, the size of the volumes of interest (VOI) or voxels will also have effect on the scale of noise. In quantitative analysis of dynamic PET data, the accuracy of kinetic parameter estimation is not only related to the signal to noise ratio, but also the number of model parameters and selection of estimation method [107].

Non-linear least square optimization is the most widely used technique to perform curve-fitting for parameter estimation in conventional compartment modeling [8, 25, 26]. The accuracy of both curve-fitting and determination of particular parameters is sensitive to selection of initial conditions [108]. If the initial conditions are improper, it becomes problematic to find a global minimum of the residual sum of squares for parameters in a multidimensional fitting space [49, 109, 110]. A rational way to obtain initial conditions is by applying parameter estimates from similar tracer kinetic studies. Likewise, species and organ dependent physiological measurements found in literature can be used to define the range for parameter boundaries. If no initial conditions are available simulation or phantom studies can be used to determine approximate values that can be used for model optimization. In addition, a number of linearizing methods can be applied to reduce computational time at the cost of limited model parameter as output [50]. Basis function and generalized linear least squares method are other options that reduce the processing time of parameter estimation compared to nonlinear least-squares approach [59, 111].

The input function has great impact on the model performance, and robust determination is crucial for the accuracy of parameter calculation [112, 113]. The input function is defined as

Table 1. Overview of kinetic modeling work with regard to hypoxia using different hypoxia PET tracers

Tracer	Image Targeting	Methods	Application	Species	Authors
^{18}F-FMISO	Hypoxia	Voxelwise kinetic modeling	Oncology/Prostate tumor	Rat	Bartlett et al. [23]
		Noninvasive pO$_2$ measurement			
^{18}F-FMISO	-	Kinetic model	Oncology	Rat	Casciari et al. [64]
^{3}H-FMISO		Monte Carlo simulations		Patient	
^{18}F-FMISO	Hypoxia, perfusion	Dynamic PET	Oncology/Brain tumor	Patient	Bruehlmeier et al. [68]
^{15}O-H$_2$O		Kinetic models			
		Logan graphical analysis			
^{18}F-FMISO	Hypoxia, perfusion	Patlak graphical analysis	Oncology/Spontaneous sarcomas	Dog	Bruehlmeier et al. [69]
^{15}O-H$_2$O					
^{18}F-FMISO	Hypoxia	Modular simulation	Oncology/Model evaluation	Simulation work	Kelly and Brady et al. [65]
		Probability density function			
^{18}F-FMISO	Hypoxia	Kinetic model	Oncology/Model evaluation	Phantom study	Wang et al. [72]
^{18}F-FMISO	Hypoxia	Pharmacokinetic analysis	Oncology/Head and neck cancer	Patient	Wang et al. [73]
		Dynamic PET/CT			
^{18}F-FMISO	Hypoxia, perfusion	Dynamic PET	Oncology/Head and neck cancer	Patient	Thorwarth et al. [70]
		Two compartment model			
^{18}F-FMISO	Hypoxia	Dynamic PET	Oncology/Radiotherapy	Patient	Thorwarth et al. [42]
		Two compartment model			
^{18}F-FMISO	Hypoxia	DCE-MRI	Oncology/Prostate tumor	Rat	Cho et al. [71]
		Dynamic PET			
		Autoradiography			
^{18}F-FMISO	Hypoxia	Dynamic PET	Oncology/Non-immunogenic carcinoma	Mouse	Bejot et al. [67]
^{18}F-3-NTR		Compartment modeling			
^{18}F-FMISO	Hypoxia	Dynamic PET	Scan protocol validation	Mouse	Whisenant et al. [66]
^{18}F-FDG		Graphical analysis			
^{18}F-FDG		Graphical analysis			
^{18}F-FMISO	Stroke	Dynamic PET	Stroke	Rat	Takasawa et al. [74]
^{18}F-FMISO	Hypoxia	Dynamic PET	Stroke	Rat	Hong et al. [75]
		Voxelwise Kinetic analysis based on basis function method			
^{18}F-FETMIN	Hypoxia	Dynamic PET	Oncology/Head and neck cancer	Patient	Lehtio et al. [80]
		Tumor to plasma ratio			
		Tumor to plasma ratio			
^{18}F-FETMIN	Hypoxia	Dynamic PET	Oncology/Head and neck cancer	Patient	Lehtio et al. [81]
		Compartment analysis			

Tracer	Target	Method	Application	Subject	Reference
^{18}F-FAZA	Hypoxia	Dynamic PET	Oncology/Squamous cell carcinoma	Mouse	Busk *et al.* [87]
		Immunohistochemical staining			
		Immunohistochemical staining			
^{18}F-FAZA	Hypoxia, perfusion	Dynamic PET	Oncology/Head and neck cancer	Patient	Shi *et al.* [88]
^{15}O-H$_2$O		Kinetic model			
^{18}F-FAZA	Hypoxia	Dynamic PET/CT	Oncology/No-small cell lung cancer	Patient	Verwer *et al.* [89]
^{60}Cu-ATSM	Hypoxia	Compartment analysis	Myocardial ischemia	Dog	Lewis *et al.* [97]
^{64}Cu-ATSM					
^{64}Cu-ATSM					
^{64}Cu-ATSM	Hypoxia	Non-steady-state kinetic simulations	Oncology/EMT6 murine carcinoma	Mouse	Holland *et al.* [100]
^{61}Cu-ATSM	Hypoxia	Electrochemical model	Oncology/Head and neck cancer	Patient	Bowen *et al.* [101]
^{18}F-FMISO		Transformation function			
		pO$_2$ microelectrode			
^{64}Cu-ATSM	Hypoxia	Kinetic modeling	Oncology/Radiotherapy	Simulation work	Dalah *et al.* [102]
^{64}Cu-ATSM	Hypoxia	Dynamic PET	Oncology/Head and neck squamous cell carcinoma	Rat	McCall *et al.* [103]
		Autoradiography			
		Immunohistochemical staining			
^{62}Cu-PTSM	Hypoxia, perfusion	Dynamic PET	Multiple tracer protocol	Simulation work	Rust *et al.* [104]
^{62}Cu-ATSM		^{62}Cu-ATSM			
^{62}Cu-PTSM	Hypoxia, perfusion	Dynamic PET	Multiple tracer protocol	Dog	Black *et al.* [105]
^{62}Cu-ATSM		Compartment analysis	Signal separation and recovery		
^{18}F-FDG	Hypoxia, perfusion and glycolysis	Dynamic PET	Multiple tracer protocol	Dog	Black *et al.* [106]
^{62}Cu-PTSM		Compartment analysis	Signal separation and recovery		
^{62}Cu-ATSM					

blood time active concentration and can be obtained either by blood sampling or noninvasively from the left ventricle or large vessels by an image derived approach [89, 113, 114]. When using arterial blood sampling the input function should be corrected for dispersion and delay. On the other hand, for the non-invasive method a number of artifacts such as partial volume effect and respiratory movements can lead to misinterpretation [115]. Additionally, correction for plasma binding and metabolites should be considered.

Besides the technical aspects of kinetic modeling of dynamic PET data there are also factors more specific related to hypoxia imaging that needs to be considered. The hypoxia tracers that have been applied in dynamic PET imaging are lipophilic compounds that enter cells by free diffusion, become metabolized and consequently trapped within the cell. Based on the proposed trapping mechanisms of used hypoxia-specific tracers it is therefore reasonable to assume that they will be unable to leave the cell for the duration of the PET acquisition. On this basis, an irreversible model will be most suitable to reflect tracer accumulation *in vivo*. However, in studies comparing different compartment models for FAZA PET the difference between reversible and irreversible two compartment models were negligible [88, 89]. Importantly, recent studies have suggested that Cu-ATSM accumulation can perhaps be influenced by copper metabolism and that there can also be an efflux of Cu-ATSM or the radioactive copper from cells [116, 117], which should be kept in mind when applying kinetic models to dynamic Cu-ATSM PET data.

The majority of studies on kinetic modeling of hypoxia PET tracers are focused on cancer. As previously mentioned perfusion limited (acute) hypoxia is caused by changes in local tumor blood flow, and regional oxygenation can therefore suddenly change. These fluctuations represent a challenge, as the estimated parameters could be average values of varying oxygenation during the dynamic PET acquisition. In order to implement the use of kinetic models in clinic, the reproducibility of model output is an important part of the validation process. The reproducibility of model parameters derived from dynamic ^{18}F-FMISO PET has been investigated and some degree of variations was observed [66]. However, part of this variation could be

due to the fluctuating nature of acute hypoxia that influence tracer uptake and potentially also impact model parameters. Moreover, because hypoxia is a heterogeneous phenomenon with microregional differences in oxygen tension within a target tissue. This represents a challenge for quantification of PET imaging as uptake in a VOI will represent an average value with contribution from multiple microregions. This will also be reflected in the parameters estimated based on TACs generated from these VOIs. As an alternative, voxel-based tracer kinetic modeling can be applied and more precisely reflects the tracer behavior in smaller subvolumes [70, 118]. However, computation time for this approach is demanding, and the error scale will be much higher, compared to parameters calculated based on TACs derived from larger VOIs. Therefore it is important to get a reasonable balance between the signal to noise ratio and the definition of voxel size and frame duration. Increasing the injected dose can be a way to improve the signal to noise ratio but this will also increase the absorbed dose and should therefore be considered with caution.

Conclusion and future perspectives

Dynamic PET based kinetic modeling represents a methodology that can potentially be used to extract additional information of cellular processes. Despite some promising results, a number of technical difficulties and limitations need to be solved for clinical implementation, e.g. the limited field of view in clinical PET scanners, and development of methods for robust determination of the input function without continuous blood sampling. Several studies have shown that it is possible to obtain kinetic parameters from dynamic hypoxia PET data but at present validation of model output against other modalities is sparse. This review points out the potential applications of dynamic hypoxia PET imaging but before a kinetic model can be fully integrated in the clinic it needs to be validated and shown that it contributes to the current assessment routine.

Disclosure of conflict of interest

None.

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