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Pharmacokinetic Analysis of Dynamic ^{18}F -FAZA PET Imaging in Pancreatic Cancer Patient

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Abstract

Purpose

This study assessed the pharmacokinetics of the hypoxia PET tracer, ^{18}F -fluoroazomycin arabinoside (^{18}F -FAZA), in pancreatic cancer (PCa) patients and determined the optimal kinetic parameters to distinguish cancerous from normal pancreatic tissue.

Method: Twenty patients with pancreatic ductal adenocarcinoma underwent dynamic ^{18}F -FAZA scans. The tissue time activity curve (TAC) was analyzed using graphical methods to determine reversibility of tracer binding and with standard compartment (S2TC) model and flow modified two tissue compartment (F2TC) model, developed to incorporate transit time of tracer through the blood vessel, to estimate the kinetic parameters. The optimal parameter set to distinguish hypoxic tumors from normal tissues was determined using logistic regression.

Results: Both graphical and kinetic model analysis indicated that tracer was reversibly bound. According to the Akaike Information Criteria, the F2TC model fitted the tumor TAC better than the S2TC model. Total distribution volume, V_T , estimated by the F2TC model for both tumor and normal pancreatic tissue was not significant but that estimated by the S2TC model was significantly different from Logan graphical analysis. The extravascular distribution volume (DV) and tracer dissociation rate constant (k_4) can classify PCa from normal tissue with sensitivity of 95% and negative predictive value of 89% ($P < 0.01$).

Conclusions: Kinetic analysis of dynamic ^{18}F -FAZA PET can distinguish PCa from normal tissue with high sensitivity. The reversibility of ^{18}F -FAZA binding in hypoxic cells could be due to glutathionylation of the nitroreductase reduced products and their subsequent efflux from same cells via the ATP mediated multidrug resistant protein (MRP-1) efflux pump.

Keywords: Hypoxia, pancreatic cancer, nitroimidazole based tracer, ^{18}F -FAZA, PET tracer kinetic modelling

Declarations:

Ethics Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Signed written informed consent was obtained from individual participants included in the study.

Consent for publication: All patients consented to their data being published

Availability of data and material: Data used in the analysis will be available from the senior author (TYL) by email tlee@imaging.robarts.ca

Conflict of interest: The authors declare that they have no conflict of interest

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Authors' contribution: FL was involved in the kinetic analysis of the images, interpretation of kinetic parameters, performed statistical analysis and wrote the manuscript. T-YL is the senior author who supervised FL in the above listed tasks. ET, IV, DAJ, DWH were involved in the study design, ethics approval, patient recruitment and study acquisition; they also approved the final manuscript.

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1. Introduction

Pancreatic cancer (PCa) ranks as the fourth most common cause of cancer death in North America because of its low overall five-year survival rate [1,2]. In 2018 alone, 55,440 Americans were diagnosed with the cancer and 44,330 died from it according to American Cancer Society [3]. Diagnosis of PCa is often made at an advanced stage after the tumor has metastasized resulting in poor survival rate [4,5]. In addition, PCa is very challenging to treat because of hypoxia induced chemo- and radio-resistance [4,6,7]. The non-invasive diagnosis of hypoxia in PCa to guide personalized treatment may improve the survival of patients.

Positron emission tomography (PET) is a non-invasive in-vivo imaging method to study the molecular and functional characteristics of cancer. A number of hypoxic tracers have been developed of which nitroimidazole (NI) based tracers, ^{18}F -fluoromisonidazole (^{18}F -FMISO) and ^{18}F -fluoroazomycin arabinoside (^{18}F -FAZA), are widely used. ^{18}F -FAZA is the preferred hypoxia tracer due to its higher lipophilic property, leading to faster delivery into cells and blood clearance and hence higher tumor to blood ratio [7–9]. In general, the tracer enters the cell through passive diffusion and the nitro group is reduced by nitroreductase to NO_2^- radical. Under well-oxygenation condition, the radical is oxidized back to its original form and diffuses out of the cells. Under poor oxygenation condition or hypoxia, the highly reactive $-\text{NO}_2$ radical damages DNA and traps the ^{18}F labelled radical. NO_2^- radical can be further reduced to hydroxylamine and its intermediates are trapped in the cells by covalently bonding to proteins and macromolecules [9–16]; normally the direct covalent bonding of NO_2^- radical to DNA is much faster than further downstream reduction via hydroxylamine [10,17]. With either route of metabolism, ^{18}F -FAZA is assumed to be irreversibly trapped in hypoxic cells (Fig. 1).

Dynamic PET provides data on the temporal distribution of a tracer in tissue, which is necessary for modelling the pharmacokinetics of the tracer [18,19]. The classical method of analysing the kinetics of NI tracer is standard irreversible two-tissue compartment (S2TC) model (Fig. 2a). One limitation of the S2TC model is that it does not model the transit of the tracer through blood vessels rather it is lumped together as the product of the tracer concentration in (arterial) blood and the blood volume. A consequence is that the estimated blood volume can be very small particularly if the dynamic PET study has a rapid frame rate (5-10 s per frame) in the first phase and the S2TC model fit includes this fast first phase. To better describe the transport of tracer into tissue, we combine the Johnson-Wilson-Lee (JWL) model [20] with the S2TC model to arrive at the flow modified two-tissue compartment (F2TC)

model. It models the flow of tracer in blood vessels and the bidirectional permeation of the blood-tissue barrier during the finite transit time through these vessels leading to a concentration gradient from the arterial end to the venous end (Fig. 2b). In contrast, S2TC model assumes the bidirectional permeation of the blood-tissue barrier occurs ‘instantaneously’ rather than over a period equals to the transit time of blood vessels.

Contrary to the common understanding of the in-vivo behaviour of NI tracers, some studies have shown that the tissue time-activity curve (TAC) is best fitted using a reversible the S2TC model [21,22]. In this study, we investigated the nature of ^{18}F -FAZA binding to pancreatic tumor in patients using graphical analysis [23] and the S2TC and F2TC model. As noted above the F2TC model does while S2TC model does not account for the fact that transport of tracer into tissue occurs over the transit time of the blood vessels rather than instantaneously, use of both the models will show how this effect affects the estimated model parameters. To confirm model fitting, forward transfer rate constant (plasma to tissue influx rate) for irreversible binding [24] or distribution volume for reversible binding [23] as calculated from the estimated S2TC and F2TC model parameters will be compared with that estimated by graphical analysis. Finally, the estimated model parameters can shed light on the possible pharmacokinetics and hence the mechanisms behind the accumulation and washout of ^{18}F -FAZA from tumor cells.

2. Methods

2.1 Patient population and image acquisition

The patient cohort consisted of 20 patients with biopsy confirmed and previously untreated pancreatic ductal adenocarcinoma. The study was approved by University Health Network Research Ethics Board and informed consent was obtained from each enrolled patient. Details of the patient population and image acquisition were described previously [25,26]. Dynamic images were acquired over 55 min with the following imaging protocol: 12@10s intervals, 8@30s, 7@120s and 7@300s in a PET/CT scanner (Discovery ST-16; GE Healthcare). Whole tumor TAC was derived from regions manually contoured by an experienced radiologist in all tumor containing tumor slices. Arterial input function (AIF) was obtained from aorta at the same level as the tumor ROIs. Out of the 20 patients, only 14 patients had TAC from normal tissue due to pancreatic atrophy in the remaining patients.

2.2 Dynamic PET analysis

Whole tumor TAC and AIF from each patient were analyzed in three ways: graphical analysis and kinetic analyses using the S2TC and F2TC model.

2.2.1 Graphical analysis

It is a compartmental analysis technique which is independent of the number and connectivity of the compartments and can be used to investigate the nature of the binding of ^{18}F -FAZA to tumor. For irreversible binding, when tissue TAC ($ROI(t)$) and AIF ($C_p(t)$) are transformed as shown in Eq (1), a linear Patlak [23,24] plot is obtained following a short delay where the slope (K_i) is the forward transfer rate constant of tracer from the blood to the bound pool and the intercept is the blood volume (V_b):

$$\frac{\int_0^t C_p(\tau) d\tau}{C_p(t)} = K_i \frac{ROI(t)}{C_p(t)} + V_p \quad (1)$$

On the other hand, Eq (2) shows that for reversible binding, the transformed $ROI(t)$ and $C_p(t)$ after a short delay are linearly related (Logan plot [23,27]) with slope equal to the sum of the extravascular distribution volume (DV) and blood volume (V_p) or total distribution volume ($V_T = V_p + V_D$):

$$\frac{\int_0^t C_p(\tau) d\tau}{ROI(t)} = (V_p + DV) \frac{\int_0^t ROI(\tau) d\tau}{ROI(t)} + \text{Intercept} \quad (2)$$

If the plot according to either Eq(1) or Eq(2) is linear, then the tracer is irreversibly or reversibly bound, respectively.

2.2.2 Standard two-tissue compartment model (S2TC):

In dynamic PET, the measured tissue activity arises from tracer in the blood vessels, free unbound tracer in extravascular space and tracer bound in the target. S2TC model categorizes these different anatomical/physiological states of the tracer as compartments. In this model, the consequence of modeling blood vessels as a compartment is that tracer once arrived is assumed to be immediately mixed uniformly with tracer already in the vessels and to immediately diffuse out to tissue. This is reflected in the flow-scaled impulse residue function ($IRF_F(t)$) where the vascular component is a delta function of area equal to the blood volume, $V_p(\text{ml} \cdot \text{g}^{-1})$. $IRF_F(t)$ is an idealized tissue TAC if the total amount of tracer is injected as a tight bolus into a blood vessel supplying the tissue of interest. The tissue TAC, $ROI(t)$ corresponding to a systemic injection of tracer as in dynamic PET is obtained by convolution of

the AIF with $IRF_F(t)$ based on the principle of linear superimposition. The above discussion is summarized by the following equations:

$$ROI(t) = C_p(t) \otimes IRF_F(t - T_0)$$

where T_0 is the delay (s) in arrival of tracers from the site where AIF is measured to the tissue region of interest

$$IRF_F(t) = \begin{cases} V_p \delta(t) & t = 0 \\ Ge^{-\alpha t} + He^{-\beta t} & t > 0 \end{cases}$$

$$\alpha = \frac{k_2 + k_3 + k_4 + \sqrt{(k_2 + k_3 + k_4)^2 - 4k_2k_4}}{2}; \beta = \frac{k_2 + k_3 + k_4 - \sqrt{(k_2 + k_3 + k_4)^2 - 4k_2k_4}}{2}$$

$$G = \frac{K_1(\alpha - k_3 - k_4)}{\alpha - \beta}; H = \frac{K_1(k_3 + k_4 - \beta)}{\alpha - \beta}$$

α, β, G and H are fitting parameters estimated from curve fitting and they are expressed in terms of the explicit model parameters K_1, k_2, k_3 and k_4 as shown above; and $\otimes$ is the convolution operator.

2.2.3 Flow modified two-tissue compartment (F2TC) model

To avoid the compartmental assumption for tracer in blood vessels with shortcomings as discussed above, we developed a new model called ‘flow modified two – tissue compartment’ model (F2TC). It models the bidirectional tracer permeation of the blood-tissue barrier during the finite transit time through blood vessels (Fig. 2b). This is reflected in the $IRF_F(t)$ where the delta function in the case of S2TC model is replaced by a rectangular function with a width equal to the transit time (w) of the tracer from arterial to venous end of blood vessels. The rest of $IRF_F(t)$ remains the same as the S2TC model. The mathematical representation for F2TC model’s $IRF_F(t)$ is:

$$IRF_F(t) = \begin{cases} F & 0 \leq t < w \\ Ge^{-\alpha(t-w)} + He^{-\beta(t-w)} & t \geq w \end{cases}$$

The fitting parameters are the same as the S2TC model except that V_p is replaced by w and can be calculated as the product of w and F according to the Central Volume Principle [28].

2.3 Analysis of Tumor and Tissue TAC

PKIN (PMOD technologies LLC, Zurich, Switzerland) with blood delay option was used to fit the S2TC model to the tumor and normal tissue TAC while custom software developed in MATLAB (The Mathworks Inc.) incorporating the

‘interior-point’ non-linear optimization routine was used to fit the F2TC model to the same TACs. The model that better represented the TACs was determined by comparing the root mean square deviations (RMSD) between the TAC and the model fit with Wilcoxon signed-rank test and by the Akaike Information Criteria (AIC [22]) for small sample size

$$AIC = N \ln \left(\frac{RMSD}{N} \right) + \frac{2(K+1)(K+2)}{N-K-2}$$

where N is the number of time frames and K is number of parameters in each model. With explicit model parameters estimated from curve fitting, important summary parameters like $K_i = \frac{k_1 k_3}{k_2 + k_3 + k_4}$, $V_D = \frac{k_1}{k_2} \left(1 + \frac{k_3}{k_4} \right)$ and $BP = \frac{k_3}{k_4}$ can be calculated. K_i is the net influx rate constant from the blood vessel to the bound pool and BP the binding potential [29,30]. V_T estimated with the F2TC and S2TC model were compared against that estimated by Logan graphical analysis by plotting the median difference and the limits of agreement as $Q1 - 1.5 * IQR$ and $Q3 + 1.5 * IQR$, where Q1 and Q3 are the first and third quartile and IQR is the interquartile range. This plot is a modification of the traditional Bland-Altman plot [31] for normally distributed data where the bias and its 95% confidence interval are plotted instead. Both the summary and explicit parameters estimated by the F2TC and S2TC model were compared using one of two non-parametric paired tests – Wilcoxon signed rank test or sign test. Depending on whether the distribution of the differences between the two sets of parameters is symmetrical or asymmetrical, either Wilcoxon signed rank test or sign test, respectively, was used to test for significant difference between the two models. Univariable logistic regression of explicit model parameters (V_P , K_1 , k_i $i=2,3,4$) and DV was used to determine their significance in differentiating normal tissue from cancer. Logistic regression with backward elimination of a group of above parameters, each selected if the associated univariable analysis attained an arbitrary chosen P-value of < 0.1 , was used to determine the optimal set of parameters to differentiate normal from hypoxic tumors.

3. Results

3.1 Reversibility of ^{18}F -FAZA Binding

The non-linear Patlak analysis plot vs the linear Logan analysis plot (Fig. 3) proved that the tracer was reversibly bound contrary to the commonly held view that it is irreversibly bound. This result was further corroborated by pharmacokinetic analysis where the root mean squared deviation (RMSD) between the model fit and measured TAC in either normal tissue or tumor was smaller with the reversible F2TC model (both α and β estimated) than the irreversible model (β set to zero) ($z = 3.78$, $p < 0.005$).

3.2 Model selection

As indicated by the AIC and RMSD in Fig. 4, our developed F2TC model was able to fit the tumor and normal tissue TAC better than the S2TC model ($p = 0.002$, $p < 0.0005$ respectively). S2TC model also estimated the blood volume (V_p) poorly. The average tumor V_p estimated by the F2TC and S2TC model, though not significantly different, was 0.1039 and 0.0737 $mL \cdot g^{-1}$, respectively, with a few S2TC model's V_p estimated to be zero which is non-physiological. According to non-parametric test, the explicit model parameters (V_p , K_1 , k_i ($i = 2,3,4$)) as well as the summary parameter BP and K_i estimated by the F2TC and S2TC model were not significantly different ($p > 0.05$). However, DV and K_1/k_2 were significantly different ($p < 0.0005$). Fig. 5 are modified Bland-Altman plots comparing V_T estimated by Logan analysis against the F2TC and S2TC model. The median differences (thick black line) and limits of agreement (dash lines) were significantly lower for the F2TC model compared to the S2TC model and V_T from the F2TC model was not but S2TC model was significantly different from that estimated by Logan analysis.

3.3 Differentiation of Tumor from Normal Tissue

Among the kinetic parameters estimated with the F2TC model, only k_4 and DV were significant ($p < 0.05$) in univariable logistic regression analysis to separate normal tissue from tumor. Using a subset of kinetic parameters (V_p , DV and k_4), each of which had $p < 0.1$ in univariable analysis, logistic regression with backward elimination identified k_4 and DV as a significant model ($p = 0.003$) to separate normal tissues from hypoxic cancerous tissues (Fig. 6a). The model correctly classified 79% of the cases with specificity of 57% and sensitivity of 95%. The positive predictive value (PPV) was 76% and negative predictive value (NPV) 89%. With the S2TC model, univariate analysis showed that only DV had $p < 0.1$ that correctly classified 71% of cases with sensitivity, specificity, PPV and NPV of 90 %, 43%, 68% and 64% respectively ($p = 0.047$).

4. Discussion

The developed F2TC model models the bidirectional permeation of the blood-tissue barrier as the tracer traverses the blood vessels over a period equals to the mean transit time, resulting in a concentration gradient from the arterial to venous end of vessels. On the other hand, S2TC model assumes that fresh tracer in arterial blood is instantaneously and uniformly mixed with tracer already in the blood vessels and instantaneously washout of blood vessels. This assumption resulted in a smaller V_P estimate than the F2TC model and in some cases even a non-physiological estimate of zero. Total distribution volume, V_T , estimated by the F2TC model for both tumor and normal pancreatic tissue was not but that estimated by the S2TC model was significantly different from Logan graphical analysis. This result was also supported by both AIC and RMSD of the fit to the tissue TAC that the F2TC model was more suited than S2TCM for describing the kinetics of ^{18}F -FAZA in hypoxic tumor and normal tissue of the pancreas.

The hypoxic pancreatic cancer tissue can be characterized from the normal tissues using k_4 and DV from the F2TC model with high sensitivity of 95% and NPV of 89%. On the contrary, DV from the S2TC model can distinguish the two tissue types with lower sensitivity and NPV. DV is a surrogate marker of SUV acquired at sufficiently long time after tracer injection, when the blood background is negligible [32]. Therefore, using DV from S2TC corroborates the usage of SUV for hypoxia imaging in the clinics, which is performed at least one hour after injection. Nevertheless kinetic analysis by providing k_4 and DV could out-perform SUV (DV) in this diagnostic task.

Graphical analysis result as well as lower RMSD from the reversible (non-zero β) F2TC model compared to the irreversible model demonstrated that the tracer ^{18}F -FAZA was reversibly bound to hypoxic PCa, contrary to the current view that NI based tracers are trapped in hypoxic cells. Unlike kinetic modelling, graphical analysis is independent of the structure (connectivity) and the number of compartments in the model which makes it more adaptable to prevailing tumor heterogeneity, i.e. a single F2TC or S2TC model may not apply to all regions in a tumor. Hence, graphical analysis is a reliable method to determine the reversibility of tracer binding. Previous studies also corroborated our finding that the kinetics of NI based tracers are best analyzed using reversible S2TC model [21,22]. Nonetheless, the mechanism behind the reversible binding of NI based tracers was not well described in the literature.

A group in Japan studied the mechanism of NI based ^{18}F -FMISO binding in nude mice by implanting cells from the human FaDu cancer line [14,33,34]. They found that the majority of the tumor radioactivity was from low molecular weight metabolite, glutathione (GST) conjugate of amino-FMISO (amino-FMISO-GH) [14,34,35]. Amino-FMISO-GH is highly hydrophilic and cannot diffuse out of the cell. However, it could efflux out via the adenosine triphosphate (ATP) dependent multi-drug resistant protein (MRP-1) [34,36], which is highly expressed in pancreatic tumor cells [5,37–39] and is responsible for drug resistance. A similar efflux of amino-FAZA-GH could explain the non-trapping of ^{18}F -FAZA in hypoxic tissue and hence the estimation of non-zero β with kinetics modelling. Since k_4 and distribution volume were comparatively larger for normal than cancerous tissue, it is likely that more amino-FAZA-GH washed out of the normal tissue leading to higher tracer accumulation and contrast between tumor and normal tissue in SUV imaging. As suggested by Masaki et al., NI based tracers may be imaging a complex processes involving nitroreductase, glutathione, and MRP-1 mediated efflux activity [34]. The tracer, ^{18}F -FAZA could be used to monitor MRP-1 activity and glutathionylation; hence could lead to personalization of treatment protocol by boosting radiation treatment in high hypoxic region and possibly treating high k_4 pancreatic cancer with MRP-1 blockers. This hypothesis warrants further investigation with more patients and different tumor types.

The major drawback of this study is that normal tissue from six patients could not be contoured due to tissue atrophy. With a complete set of normal data, the sensitivity and specificity could improve. The measurement of oxygen partial pressure in the tumor of this group of patients was not done as the approved ethics protocol did not include this invasive procedure. Nevertheless, pancreatic glands in PCa are surrounded by dense desmoplastic reaction for the survival of the cancer cells [40]. The high sensitivity (95%) in distinguishing the tumor from normal tissue agrees with the current view that pancreatic tumor is highly hypoxic due to this prevalent desmoplasia and the tracer ^{18}F -FAZA is a specific nitroreductase substrate in hypoxic cells. Furthermore, normal tissue neighbouring PCa may be relatively hypoxic compared to that in normal pancreas owing to the dense mass of fibrogen and collagen from desmoplasia. This could explain the low specificity observed in separating tumor from normal tissue.

5. Conclusion

We have developed the flow modified two tissue compartment (F2TC) model to analyze the kinetics of the hypoxic tracer ^{18}F -FAZA in pancreatic cancer. Using the F2TC model, the estimated distribution volume (DV) and dissociation rate constant (k_4) of the tracer were able to distinguish pancreatic cancer from normal tissue with high sensitivity (95%) and high negative predictive value (89%). In this paper only 20 patients were analyzed, and larger N is worth investigating. Our result also showed that ^{18}F -FAZA was not irreversibly trapped in the putative hypoxic pancreatic cancer cells because the glutathione conjugated nitroreductase reduced product can exit hypoxic cells via the MRP-1 efflux pump.

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Figure Legends

Figure 1. Current view of ^{18}F -FAZA binding in cells under condition of normoxia and hypoxia.

Figure 2. a) S2TC model: free unbound tracer in the blood vessel and extravascular extracellular space (EES) and the bound tracer in intracellular space (ICS) are categorized into different compartments. $K_1 (\text{mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1})$ is the tracer influx rate constant into EES, $k_2 (\text{min}^{-1})$ is the efflux rate constant from EES to blood, $k_3 (\text{min}^{-1})$ is binding rate constant, and $k_4 (\text{min}^{-1})$ is disassociation rate constant. If tracer binding is irreversible, k_4 is zero. (b) F2TC model: The EES and ICS compartment are retained as in S2TCM but blood vessels are represented as a cylindrical tube, and as tracer traverse the blood vessels, it diffuses into EES creating a concentration gradient from arterial to venous end. $F (\text{mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1})$ is blood flow. The corresponding flow scaled impulse residue function (IRF_F) is shown below each model.

Figure 3. Graphical analysis of ^{18}F -FAZA TAC from a pancreatic tumor : Linear plot of Logan analysis (a) and non-linear plot of Patlak analysis (b) indicated that the tracer was reversibly bound contrary to the current view of irreversible binding.

Figure 4. Comparison of F2TC and S2TC model in fitting tumor and normal tissue TAC using (a) RMSD and (b) AIC as measure of goodness of fit. The x-axis is patient number. Case #1-20 corresponds to tumors and Case #21-34 are normal pancreatic tissues from same patients as 1-20. Normal tissues were observable in PET imaging in 14 patients only.

Figure 5. Modified Bland-Altman plot comparing total distribution volume (V_T) estimated for hypoxic and normal tissue with Logan graphical analysis and with (a) F2TC and (b) S2TC model. The solid lines are the median differences and dashed lines are explained in the text.

Figure 6. Distinguishing pancreatic tumor from normal tissue using kinetic parameters estimated with F2TC and S2TC model. (a) For the F2TC model, distribution volume (DV) and k_4 can distinguish the two tissue types with sensitivity of 95% and (b) For the S2TC model, DV achieved a sensitivity of 90%. The solid line in each case is the linear discriminator derived from the Youden index (Cancer 1950; 3(1): 32-35). For each case, DV for one patient's

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Figure 7. Mechanisms for hypoxia imaging with nitroimidazole based tracers. The region indicated by dashed box is the proposed mechanisms behind the reversibility of tracer binding (adapted from Ref. 14).

Figures

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Mechanisms for hypoxia imaging with nitroimidazole based tracers. The region indicated by dashed box is the proposed mechanisms behind the reversibility of tracer binding (adapted from Ref. 14).