Published in final edited form as:

Phys Med Biol. 2014 December 21; 59(24): 7937–7955. doi:10.1088/0031-9155/59/24/7937.

Heterogeneity in stabilization phenomena in FLT PET images of canines

Urban Simoncic^{1,2,3} and Robert Jeraj^{1,2,3}

¹Jozef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia

²Medical Physics Department, University of Wisconsin – Madison, 1111 Highland Avenue, Madison, WI 53705–2275, USA

³The Centre of Excellence for Biosensors, Instrumentation and Process Control – COBIK, Tovarniska 26, 5270 Ajdovscina, Slovenia

Abstract

3'-(¹⁸F)fluoro-3'-deoxy-L-thymidine (FLT) is a PET marker of cellular proliferation. Its tissue uptake rate is often quantified with a Standardized Uptake Value (SUV), although kinetic analysis provides a more accurate quantification. The purpose of this study is to investigate the heterogeneity in FLT stabilization phenomena.

The study was done on 15 canines with spontaneously occurring sinonasal tumours. They were imaged dynamically for 90 min with FLT PET/CT twice; before and during the radiotherapy. Images were analyzed for kinetics on a voxel basis through compartmental analysis. Stabilization curves were calculated as a time-dependant correlation between the time-dependant SUV and the kinetic parameters (voxel values within the tumour were correlated). Stabilization curves were analyzed for stabilization speed, maximal correlation and correlation decrease following the maximal correlation. These stabilization parameters were correlated with the region-averaged kinetic parameters.

The FLT SUV was highly correlated with vasculature fraction immediately post-injection, followed by maximum in correlation with the perfusion/permeability. At later times post-injection the FLT SUV was highly correlated (Pearson correlation coefficient above 0.95) with the FLT influx parameter for cases with tumour-averaged SUV_{30–50min} above 2, while others were indeterminate (correlation coefficients from 0.1 to 0.97). All cases with highly correlated SUV and FLT influx parameter had correlation coefficient within 0.5% of its maximum in the period of 30–50 min post-injection. Stabilization time was inversely proportional to the FLT influx rate. Correlation between the FLT SUV and FLT influx parameter dropped at later times post-injection with drop being proportional to the dephosphorylation rate. The FLT was found to be metabolically stable in canines.

First and corresponding author: Urban Simoncic, Institute Jozef Stefan, Jamova 39, SI-1000 Ljubljana, Slovenia, urban.simoncic@ijs.si.

Conflict of Interest Notification

There are no conflicts of interests.

FLT PET imaging protocol should define minimal and maximal FLT uptake period, which would be 30–50 min for our patients. Additionally, kinetic analysis should be used when low FLT avidity is expected. Low SUVs should be treated with great caution.

Keywords

FLT; PET; image quantification; Kinetic analysis; uptake period

Introduction

3'-(¹⁸F)fluoro-3'-deoxy-L-thymidine (FLT) is a marker of cellular proliferation that is used for PET/CT imaging (Shields *et al* 1998). FLT uptake into the tissue can be modelled with a two-tissue compartment, five-parameter kinetic model consisting of four rate parameters and one parameter for the blood volume fraction (Muzi *et al* 2005a). The macroparameter $K_i = K_1 k_3 / (k_2 + k_3)$, also known as the FLT tissue influx rate, quantifies the rate of FLT uptake into the tissue, which is indirect measure of DNA synthetic rate and cellular proliferation (Mankoff *et al* 1998, Muzi *et al* 2005a). In addition to the FLT influx rate, other kinetic parameters may be clinically useful (Jeraj *et al* 2010). Instead of doing full kinetic analysis, the FLT tissue influx rate can be quantified with the Standardized Uptake Value (SUV), which is a semi-quantitative measure of the radiopharmaceutical tissue uptake, calculated by correcting the single static PET image for the injected activity and the size of the imaged subject (Huang 2000, Thie 2004). Multiple SUV's at different times post-injection could potentially serve as surrogate markers for other kinetic parameters, as proposed for 2-deoxy-2-(¹⁸F)fluoro-D-glucose (FDG) that have similar uptake kinetics as FLT (Strauss *et al* 2003). However, the SUV is not a very reliable surrogate marker even for the tracer's tissue influx rate due to variability in the available tracer in the plasma as an inherent limitation of the SUV (Keyes 1995).

Besides this inherent limitation of the SUV, additional uncertainties could arise due to image acquisition at suboptimal time point. For the FDG PET imaging as the most common PET imaging, influence of uptake period on the quantification is well investigated and summarized in recommendations by SNM (Delbeke *et al* 2006), EANM (Boellaard *et al* 2010), EORTC (Young *et al* 1999), PERCIST rules (Wahl *et al* 2009) and NCI guidelines (Shankar *et al* 2006) that suggest the uptake period in the range from 45 min to 70 min. FLT PET imaging is less mature and no such recommendation exists yet for this tracer. Typical uptake period in studies involving FLT PET/CT ranges from 30 min (Muzi *et al* 2005a, Muzi *et al* 2006, Price *et al* 2009, Contractor *et al* 2011) to 90 min or more (Dittmann *et al* 2003, Smyczek-Gargya *et al* 2004).

In addition to inadequate evidence that could suggest the appropriate uptake period for FLT PET imaging, published correlation coefficients between the FLT SUV and FLT influx rate parameter are rare and differ considerably. Relatively high correlation coefficient (0.91) has been found for the head and neck cancer patients (Menda *et al* 2009). Similar correlation coefficients (0.86 and 0.90) using early (10–25 min) and late (50–60 min) SUVs were found for recurrent high-grade glioma patients (Schiepers *et al* 2010). On the other hand, low

correlation coefficients of 0.71 and 0.62 have been reported for gliomas (Muzi *et al* 2006) and lung cancer (Muzi *et al* 2005b), respectively.

Main purpose of this study was to investigate the stabilization of FLT tissue uptake by determining when and to what extent the SUV represent FLT influx rate or other clinically relevant model parameter. In addition, this study aimed to find the relations between the FLT tissue uptake stabilization parameters (i.e. characteristic post-injection periods when the SUV represent investigated model parameters, degree of agreement between the SUV and investigated model parameters) and region-averaged model parameters. Motivation for these analyses was to help finding optimal post-injection periods for static FLT PET imaging and to properly interpret resulting FLT SUV images.

Methods and materials

FLT tissue uptake model

Similarly to the thymidine in plasma, FLT is transported from the plasma to the intercellular matrix and from there into cells. Intracellular FLT is metabolized to FLT-monophosphate, FLT-diphosphate and FLT-triphosphate, but it is not incorporated into DNA. Appropriate model for the FLT tissue uptake is two-tissue compartment, five-parameter kinetic model consisting of four rate parameters and one parameter for the blood volume fraction. The FLT concentration in the plasma serves as model input function, freely exchangeable FLT in the tissue forms first compartment and phosphorylated FLT forms second compartment (Muzi *et al* 2005a). Model assumes that the FLT transport rates between the compartments are proportional to the concentrations in the source compartments, so it is described with differential equations in Eq. 1:

$$\begin{aligned}\frac{dC_e(t)}{dt} &= K_1 I_p(t) + k_4 C_m(t) - (k_2 + k_3) C_e(t) \\ \frac{dC_m(t)}{dt} &= k_3 C_e(t) - k_4 C_m(t)\end{aligned}\quad (1)$$

The FLT activity concentration in the plasma is denoted as $I_p(t)$ and serves as an input function for the model, whereas the activity concentrations in the first and second compartment are denoted as $C_e(t)$ and $C_m(t)$, respectively. Parameters K_1 , k_2 , k_3 and k_4 are model kinetic parameters. Macroparameter $K_i = K_1 k_3 / (k_2 + k_3)$, also known as FLT tissue influx rate, is a measure of the tissue uptake rate of FLT and is correlated to the cellular proliferation labelling index Ki-67 (Vesselle *et al* 2002, Muzi *et al* 2005b, Shinomiya *et al* 2013). The activity concentrations measured by PET scanner are contributed by both compartments and vasculature activity. Therefore, the $tTAC(t)$ (total Time Activity Curve) as a model-prediction for PET measurement contains an additional parameter V_b that accounts for the vasculature in the tissue (Eq. 2).

$$\begin{aligned}C_i(t) &= C_e(t) + C_m(t) \\ tTAC(t) &= C_i(t) + V_b I_b(t)\end{aligned}\quad (2)$$

The $I_b(t)$ is whole-blood activity concentration. The solution of FLT model from Eq. 1 and Eq. 2 is given in the Appendix, together with the relevant simplification of the model.

Subjects and data acquisition

The study was done on 15 canine veterinary patients with spontaneously occurring sinonasal tumours (8 carcinomas and 7 sarcomas) without undergoing previous therapy. Patients were imaged dynamically with FLT PET/CT before the start of radiotherapy and once again during the course of fractionated radiotherapy. Anaesthesia and immobilization with a patient specific bite-block, dental mould, and vacuum mattress provided superior immobilization and ensured sub-millimetre reproducibility. Therefore, the signal-to-noise ratio (SNR) on the voxel basis was higher than obtainable in human patients. Patients were injected with FLT when positioned on scanner table. Injection was manual into a line connected to the cephalic vein. Injected activity was 5–10 mCi and injection volume was 0.6–10.0 mL. The duration of the FLT bolus was up to 10 s and was followed by flushing the injection line with 10 mL of saline solution. A 90 min dynamic PET scanning over a 30 cm FOV started simultaneously with the administration of the FLT. During the dynamic PET imaging blood samples (1 mL) were withdrawn from contralateral cephalic vein at 5, 15, 30 and 90 min post-injection for the first two patients for metabolite analysis.

Canines were referred to the University of Wisconsin Veterinary Medical Teaching Hospital. The research protocol was approved by the Animal Care and Use Committee of the University of Wisconsin, and all canine owners signed a written informed consent form. The data were collected in the Clinical Imaging Facility at the University of Wisconsin - Madison. FLT was produced on-site using the UW Comprehensive Cancer Center Translational Imaging Research Core's Radiopharmaceutical Production Facilities. Canine patients were scanned with GE Discovery VCT PET/CT scanner and reconstructed to $128 \times 128 \times 47$ voxels.

Analysis

Standardized Uptake Value—Specific activity images acquired during PET imaging were transformed into SUV images by normalizing to the injected activity and multiplied by the patients' weights. Average SUV from 30 to 50 min post-injection ($SUV_{30-50min}$) was used in analysis, where the SUV parameter with proper uptake period is used.

Input function extraction—Input functions were extracted from dynamic PET images. Carotid artery and nearby background region were manually delineated for each dynamic PET image on the early PET frame, when the vasculature was visible. Segmented dynamic PET images were decomposed into two time activity curves by factor analysis with two factors. One factor corresponded to the whole-blood time signal and another to the tissue time signal. Which factor corresponding to the whole-blood time signal was determined visually, based on the shape of both time signals. When blood sampling was available, whole-blood time signal was scaled to fit the blood samples at 30 min and 90 min. When blood sampling was not available, average scaling factor from patients with blood sampling was applied. Factor analysis was implemented in Matlab (The Mathworks, Natick, MA, USA) as a least-squares-based problem with non-negativity constraints for factors and factor images.

FLT metabolite analysis—Two patients had pre- and mid-therapy blood samples analyzed for metabolites through rapid solid-phase-extraction method. Analysis was done C-18 Sep-Pak cartridges (Waters Corporation, Milford, MA) that were rinsed with water and ethanol to separate the polar metabolites like FLT-glucuronide from parent FLT (de Langen *et al* 2009). Blood samples were centrifuged for 10 min to separate blood plasma from whole blood. A 100 μ L of blood plasma were diluted with 5mL of deionized water. Sep-Pak cartridge was prepared by rinsing with 10 mL of ethanol, followed by 10 mL of deionized water. Plasma solution was filtered through recently prepared Sep-Pak cartridge and collected in “metabolite” test tube. Sep-Pak was rinsed with additional 5 ml of deionized water, which was collected in the same “metabolite” test tube. The cartridge was then rinsed with 10 mL of 96% ethanol that was collected in a “parent FLT” test tube. Test tubes were measured on the gamma counter and the percentage parent within each plasma sample was calculated.

Kinetic analysis—The dynamic FLT PET images were analyzed for kinetics using the above described compartmental model on a *voxel basis*. The k_2 parameter was substituted with early volume of distribution $V_d = K_1/k_2$ in the process of parameter optimization and vasculature fraction V_b was imposed as model parameter. The influx rate constant $K_i = K_1 k_3 / (k_2 + k_3)$ was calculated from the estimated parameters. Kinetic analysis was done with custom-developed program in Matlab, which utilizes a cumulative representation of the input functions (Simoncic and Jeraj 2011) that simplifies the kinetic analysis implementation and improves its accuracy when image-derived input function is used.

Calculation of FLT PET stabilization curves—The time course of the relationship between individual parametric image and the time-dependant SUV was assessed with a correlation analysis: Pearson correlation coefficients were calculated between the SUV of each individual time frame and the parametric image. This time-dependant correlation coefficient that was evaluated at discrete time points was considered as the stabilization curve for the kinetic parameter (Simoncic and Jeraj 2007). Similar methodology has been applied for kinetic parameters for FDG (Strauss *et al* 2003) and for macroparameter K_i from Patlak analysis for FLT (Menda *et al* 2009). However, Strauss *et al* and Menda *et al* evaluated stabilization curves on the population level. In other words, they evaluated time-dependant correlation coefficient between the kinetic parameters evaluated for multiple tumour regions and corresponding time-dependant region-averaged SUV. In that way they got single stabilization curve for the population of patients.

By our methodology, stabilization curves are obtained as time-dependant correlation coefficient between the kinetic parameters for voxels within the tumour and time-dependant SUV evaluated on the same voxels. Therefore, single stabilization curve was obtained for each tumour and each imaging session separately. Calculation of the stabilization curves for each individual patient and imaging session is sensitive to the noise and motion artefacts. Canine patients had good SNR due to high injected dose and virtually no motion artefacts due to tight fixation and anaesthesia. In order to further increase SNR, parametric images and dynamic FLT PET/CT images were downsampled to $64 \times 64 \times 24$ voxels.

Tumours of interests were segmented based on FLT PET/CT image by veterinarians. For the evaluation of stabilization curve, time dependant correlation coefficients between the kinetic parameters and time dependant SUVs were calculated for those voxels that were inside the tumour segment.

Estimation and analysis of stabilization parameters—Out of all kinetic parameters, most clinically relevant parameters are K_i , K_I and V_b , as they measure the FLT tissue influx rate (cellular proliferation rate), blood perfusion/vessel permeability, and vasculature fraction in the tissue, respectively. Therefore, stabilization curves for these kinetic parameters were analyzed. Exemplary stabilization curves are in Figure 1a for K_i parameter and in Figure 1b for K_I or V_b parameters. The K_i stabilization curve is initially low, then it rises until it reaches maximum, and decreases after the maximum. The K_I or V_b stabilization curve is high at the start of scan and decrease with time.

Stabilization curves were analyzed by introducing the concept of stabilization parameters. For the K_i stabilization curve, three stabilization parameters were defined: time-to-stabilization (*TTS*), maximal correlation (*MC*) and corrected drop from correlation maximum to the end of scan (*CD*). For K_I and V_b stabilization curves, only the *TTS* was assessed.

Stabilization parameters were estimated through the areas over/under the curve that are defined in Figure 1. The K_i stabilization parameter *TTS* (meaning time-to-stabilization) has units of time and was defined as $A' / (\rho'_{max} - \rho'_{min})$. The *TTS* is a characteristic stabilization time that is generally much shorter than the time to reaching maximum and is analogous to the mean lifetime in exponential decay. Both the *TTS* and mean lifetime are defined (up to scaling factor) as the area under/over the curve, divided by the interval that curve's dependant value can reach. However, the *TTS* cannot be interpreted as the time when stabilization curve crosses certain level, like the mean lifetime in exponential decay. The *TTS* is only a measure of how fast the stabilization curve approaches to its maximum. The parameter *MC* was defined as the maximal correlation or ρ'_{max} . The parameter *CD* is a measure of how much the correlation drops from the maximum by the end of imaging interval, and was defined as $A'' / (t'_{max} - t'_{min})$. The *TTS* for K_I and V_b parameters were defined as $A / (\rho_{max} - \rho_{min})$; i.e. in the same way as for K_i parameter, except that area *A* was used instead of *A'*, and similarly minimal and maximal correlations ρ_{min} and ρ_{max} .

Statistical analysis—Stabilization parameters for individual's stabilization curves were assessed for their association with the tumour-average kinetic parameters by calculating the correlation coefficients between the stabilization parameters (or their inverse in the case of *TTS*) for all the cases and their tumour-average kinetic parameters. Time range when the stabilization curve is above certain threshold was evaluated for the cases with maximal SUV(t)– K_i correlation above 0.9. Thresholds were defined as: (1) fixed correlation coefficient of 0.9, (2) correlation decreased for no more than 2% from the maximum, and (3) correlation decreased for no more than 0.5% from the maximum. All analysis was done using Matlab (The Mathworks, Natick, MA, USA).

Results

Temporal evolution of the FLT SUV

Early SUV was similar to the V_b parametric image, accompanying or closely followed by the SUV that was similar to the K_I parametric image. In most cases, early SUV was always more similar to V_b than K_I parametric image. At later times (30+ min) the SUV was usually similar to the K_i parametric image. The behaviour is illustrated by an exemplary image in Figure 2.

Stabilization curves for individual FLT PET images

Stabilization curves for K_i , K_I and V_b kinetic parameters were unique for each patient, but they shared some common characteristics. Namely, the maximum in V_b stabilization curve appeared first and was accompanied or followed by the maximum in K_I stabilization curve. The SUV at late time post-injection was highly correlated to the FLT tissue influx parameter K_i , with possible decrease in correlation, after the curve reached maximum. Typical example of stabilization curves for K_I , V_b and K_i parameters is in Figure 3.

The majority of cases qualitatively followed the presented example in Figure 3. There were only 7 out of 30 cases with an early time point SUV being highly correlated with K_I (i.e. correlation 0.9 or above) and at the same time the correlation between SUV and V_b being lower than the correlation between the SUV and K_I by 0.1 or more. So, for great majority of cases, the early SUV was not good indicator for K_I parameter at any time point. In addition, a few cases did not follow the presented example in Figure 3 even qualitatively. Figure 4 presents two notable exceptional cases. Example a) is one of those 7 cases, where the early SUV was good indicator for K_I , but this case is notable also because of very slow FLT uptake stabilization. Example b) is notable for almost no correlation between the SUV(t) and K_i at any time point.

Heterogeneity in the FLT PET stabilization

Despite the common characteristics in FLT tissue uptake stabilization phenomena, quantification of stabilization parameters showed great heterogeneity. Statistical summary of the stabilization parameters is in Table 1.

Stabilization parameters were correlated with the average kinetic parameters in the patient's tumour. The TTS^{-1} for K_i stabilization curve was highly correlated to the tumour-averaged k_3 and K_i parameters, which means that the *tumours with higher uptake rate stabilize faster*. The MC was also notably correlated with the tumour-averaged k_3 and K_i parameters, and the CD was highly correlated to the tumour-averaged k_4 parameter. No significant correlation was found between the TTS^{-1} for K_I or V_b parameters and tumour-averaged kinetic parameters. Correlation coefficients between the stabilization parameters and tumour-averaged kinetic parameters are in Table 2.

Correlation plots of the TTS^{-1} for K_i stabilization curve versus the tumour-averaged k_3 and K_i parameters (parameters with the highest correlation) are shown in Figure 5. Both plots in

Figure 5 confirm a highly linear relation between the TTS^{-1} for K_i stabilization curve and selected kinetic parameter.

Correlation plots of the MC versus the tumour-averaged k_3 and K_i parameter are shown in Figure 6. These correlation plots disclose that the relation between the parameter value (either k_3 or K_i) and MC may not be linear as there are outliers with unusually low MC .

Correlation plot of the CD versus the tumour-averaged k_4 parameter is shown in Figure 7. This correlation plot shows linear relation between the k_4 parameter value and the CD .

SUV-Ki correlation versus tumour-averaged SUV_{30–50min}

Tumour-averaged SUV_{30–50min} ranged from 0.79 to 4.7. Mean and median values were 1.7 and 1.4, respectively. Maximal correlation (MC) between the SUV(t) and K_i was almost unity (0.95 to 1.0) for tumours with tumour-averaged SUV_{30–50min} above 2. Tumours with tumour-averaged SUV_{30–50min} below 2 had variable MC – three had 0.94 or more, while eighteen had it below 0.85 with the lowest being 0.11. MC versus the tumour-averaged SUV_{30–50min} is in Figure 8.

Based on Figure 8, tumours can be arbitrarily subdivided into two groups: (1) group with high SUV– K_i correlation having MC above 0.9 and (2) group with low to medium SUV– K_i correlation having MC up to 0.9. Obviously, time range for the SUV(t)– K_i correlation above certain threshold is interesting only for high SUV– K_i correlation group, so these time ranges for three correlation thresholds are reported in Table 3. Thresholds for the SUV– K_i correlation were set to correlation above 0.9 ($\rho > 0.9$), correlation decreased for no more than 2% from the maximum ($\rho > 0.98 \times MC$) and correlation decreased for no more than 0.5% from the maximum ($\rho > 0.995 \times MC$).

Patients in the high SUV– K_i correlation group had SUV(t)– K_i correlation above 0.9 for a time period starting early post-injection (3 – 19 min), and persisted until the end of scan at 90 min. The SUV(t)– K_i correlation was within 2% of its maximum in time period starting on average 15 min (range: 10 – 23 min) post-injection, and persisted at least to 61 min post-injection, while it lasted until the end of scan for 7 out of 9 tumours. For all the tumours, the SUV(t)– K_i correlation was within 0.5% of its maximum in time period from 30 to 50 min post-injection.

Blood metabolite analysis

Fraction of FLT metabolites in blood plasma was below 5%, irrespective of patient and time point. All the metabolite measurements are in Table 4. Because all four measurements have shown that the FLT was not metabolized, no further analysis of metabolites was done.

Discussion

Shape of the stabilization curves

The FLT uptake stabilization for individual patients shared some common characteristics; namely the highest correlation for V_b immediately post-injection, accompanied or followed by the highest correlation for K_i that was in most cases lower than the correlation for V_b ,

and high correlation between the late SUV and K_i that was also not seen in all the cases. The result is not completely new because the similar behaviour on the population level for FDG patients was already observed (Strauss *et al* 2003).

The shape of V_b and K_I stabilization curves could be related to the sensitivity functions, which represents time-dependent percentage change in FLT tissue uptake model output resulting from 1% change in parameter value. Muzi *et al* reported sensitivity functions for microparameters of FLT tissue uptake model for three characteristic parameter sets representing tumour, muscle and bone marrow (Muzi *et al* 2005a). The FLT tissue uptake model output showed the highest sensitivity to K_I parameter in first 10 min post-injection for all three tissue types. This article however did not report the sensitivity functions for V_b parameter, which has sharp peak immediately post-injection (before the peak of the K_I sensitivity function), followed by rapid decrease to the value close to zero (Ullrich *et al* 2008). The shape of V_b sensitivity function by Ullrich *et al* and the shape of K_I sensitivity function by Muzi *et al* therefore correspond to the shapes of V_b and K_I stabilization curve at least qualitatively, which seems to explain the stabilization curves' shapes. However, the sensitivity functions depend on the set of kinetic parameters that are used as the model parameters; i.e. which parameters are kept constant while one is perturbed. Muzi *et al* used K_I , $V_d=K_I/k_2$, k_3 and k_4 as the model parameters (Muzi *et al* 2005a), while Ullrich *et al* used K_I , k_2 , k_3 and k_4 parameters (Ullrich *et al* 2008). By trying both combinations we found that selection of model parameters is primary reason for significant difference between the Muzi's K_I sensitivity function and Ullrich's K_I sensitivity function; first has maximum a few minutes post-injection and then decreases, while second asymptotically approaches to one. In addition, the sensitivity function for macroparameter K_i cannot be evaluated in the same way because macroparameter is a function of microparameters and it cannot be perturbed while keeping all other model parameters unchanged. After all, we cannot expect that only one kinetic parameter changes across the tumour, while others would be unchanged. Simultaneous variation of parameters across the tumour and difference in the K_I sensitivity functions due to different ways of their evaluation are good indicator that sensitivity functions are not sufficient explanation for the shapes of the stabilization curves. They can be better explained by exploring the FLT tissue uptake model equations.

Initial high correlation between the SUV and the V_b parameter is obvious from the second equation in Eq. 2, knowing that the blood activity concentration is the same for all the voxels, whereas the tissue activity at the time of injection is negligible ($C_i(t=0)=0$). A high correlation between the SUV and the K_I parameter shortly after the injection follows from the Eq. 3. Assuming the $t \rightarrow 0$ in the Eq. 3, the $IRF(t \rightarrow 0)=K_I$ and the tissue activity shortly after the injection is proportional to the parameter K_I . The SUV long time post-injection is correlated to the FLT influx rate (K_i parameter) if the backflow from the terminal compartment (k_4 parameter) is low enough. Firstly, the activity concentration in the blood is much lower than the activity concentration in the tissue (unless the FLT avidity is very low), so the activity concentration in the blood does not affect the correlation significantly and we can assume $tTAC(t)=C_i(t)$. Secondly, under the assumption of low k_4 parameter value (i.e. $k_4 \ll k_2+k_3$), the $IRF(t)$ and the $C_i(t)$ for constant input function are in Eq. 4 and Eq. 5, respectively. The tissue activity concentration curve with any realistic input function would

be something in-between the tissue activity concentration curve for impulse and constant activity in the plasma, as derived in Eq. 6 and further simplified in Eq. 7. Therefore, the $tTAC(t)$ at late time post-injection is generally determined by the influx parameter $K_i = K_1 k_3 / (k_2 + k_3)$, albeit it may depend on time and could be affected by some corrections that are not negligible.

Heterogeneity in the FLT PET stabilization

Significant correlation of the TTS^{-1} for K_i stabilization curve with the k_3 parameters can be explained with the model for the FLT tissue uptake. First, we need to explain the reasons for investigating the TTS^{-1} , not the TTS itself. The TTS have similar meaning as the mean time in exponential decay, implying that the higher TTS indicates slower transient phenomena. On the other hand, the simplified solution of two-tissue compartment, four-parameter kinetic model (Eq. 4) indicates that the higher kinetic parameters k_2 and k_3 should result in faster transient phenomena, so positive correlation between the TTS^{-1} and kinetic parameters k_2 and k_3 can be expected. However, the significant correlation was observed only for the k_3 parameter, not for the k_2 parameter. This might seem unexpected, since the model equations suggest there is a transient phenomenon in image stabilization that is having a functional form $\exp[-(k_2 + k_3)t]$. Here we have to note that these equations include the term $k_3 + k_2 \times \exp[-(k_2 + k_3)t]$, which imply that an increase in the k_3 parameter will increase the relative importance of the k_3 versus the k_2 term. Both of these effects would contribute to a higher correlation between the K_i and SUV. On the other hand, if the k_2 parameter is increased relative to the k_3 , this will decrease the exponential $\exp[-(k_2 + k_3)t]$ and increase the relative importance of k_2 versus the k_3 ; these effects will partially cancel out, leading to a smaller dependence on k_2 for the correlation between the K_i and SUV.

The observed correlation between the TTS^{-1} for K_i stabilization curve and the average K_i parameter was even higher than for the k_3 parameter, which might be because of combination of two reasons. The K_i parameter is calculated from the k_3 parameter so the K_i and k_3 parameters are correlated, which explain some correlation, but not the highest correlation. In addition, the estimate for a macroparameter K_i is generally more stable and has lower error, when comparing to the estimates of internal model parameters like k_3 . Therefore, the highest correlation between the TTS^{-1} for K_i stabilization curve and the average K_i parameter might be explained by the combination of (1) correlation between the K_i and k_3 parameters and (2) innate greater stability and lower error of the estimate for a macroparameter like K_i versus the estimates for internal model parameters.

It was also observed that some K_i stabilization curves decreased at late time post-injection. The CD was correlated with the average k_4 parameter. The reason for the impaired correlation between the SUV and K_i at late time post-injection is non-negligible backflow from the terminal compartment; i.e. the k_4 parameter is not negligible, although the relation $k_4 \ll k_2 + k_3$ still holds and the approximations in Eq. 4 to Eq. 7 are still valid. When the k_4 parameter is not negligible, the tissue specific activity at late time post-injection is reduced. The reduction at particular time point is proportional to the k_4 parameter and is increasing with time (Eq. 5), which explains the decrease of stabilization curves at late time post-injection and its correlation with the average k_4 parameter.

It was unexpected that no correlations were found between the TTS^{-1} for K_I or V_b and average kinetic parameters. That might be because the fading out of K_I or V_b information is more complex and cannot be summarized with the linear function of single kinetic parameter. It is also possible that the method of TTS estimation through the area under the curve is too primitive to give any meaningful result in the case of K_I and V_b . We have to note that the TTS values for K_I and V_b parameters would be different if the difference between maximal and minimal values changed. Minimum and maximum in stabilization curve are affected by various factors, especially all sources of uncertainty and particular spatial distribution of kinetic parameters.

The use of TTS to measure how fast the FLT uptake stabilizes might seem contrainuitive as the time of curve's extreme would be an optimal time for imaging. However, for the selection of uptake period is not important just the time of maximum correlation, but also how fast the correlation reaches value close to maximum. With the aim to cover also that aspect, we defined TTS through the area over the curve. The TTS as defined is affected by the time of curve's extreme and also by the shape of the curve; if two stabilization curves reached their extreme at the same time, the TTS would be lower for the curve with steeper increase in early period and more gradual increase in later period (i.e. the curve that reaches values close to maximum sooner). Similarly to the TTS , the CD could be defined simply as the drop of correlation from the maximal value. With the CD definition through the area over the curve, the CD is affected by the drop of correlation from its maximum and the shape of the stabilization curve.

Shape of the stabilization curves is most likely affected by the FLT injection duration, which was manual and therefore not perfectly reproducible. However, the injection was quick, so the duration of injection varied for seconds, not more. This might affect the input function peak, but not the plateau. So, it can have some effect on the correlation curve immediately post-injection, but not at the later times. Therefore, the V_b and K_I stabilization parameters may be affected by the injection schedule, which might be one of the reasons why no correlations of these parameters with regional kinetic parameters were found. K_i stabilization curves at later times, however, should be insensitive to the injection schedule.

Another factor that generally affect the SUV quantification is the definition of SUV; in particular the SUV definition of body habitus (weight, lean body mass, or body surface area). Although different definitions can affect the SUV quantification considerably, it should be noted that the SUV scaling has no impact on the stabilization curves, because the intra-tumour correlation of FLT SUV with kinetic parameters was calculated. Therefore, the multiplication of SUV with some constant because of different body habitus used does not affect the correlation at all. In contrary to that, SUV definition of body habitus would make difference if inter-patient correlation of FLT SUV with kinetic parameters was calculated (Strauss *et al* 2003, Menda *et al* 2009). Although the stabilization curves would not be affected by the SUV definition of body habitus, different definition would change the SUV threshold below which the SUV would be considered unreliable.

Image-derived input function was not corrected for metabolites and plasma to whole-blood ratio and scaled with venous blood samples or average scaling factor. Absence of metabolite

correction was supported by measured metabolites in four patients/imaging sessions, with observed fraction of FLT metabolites in blood plasma varied over time from 1% to 3%. That amount of FLT metabolites in blood plasma can be safely neglected in kinetic analysis. While the negligible FLT metabolite fraction in blood plasma has not been reported yet for canines, it has been observed in mice tumour models (Barthel *et al* 2003, Kim *et al* 2008). Assumption that parent plasma FLT activity is equal to the whole-blood activity was based on the statistically insignificant differences between plasma and whole-blood specific activities found in humans (Visvikis *et al* 2004). Direct or indirect scaling of input function with venous blood samples is supported by some clinical evidence in humans; e.g. the use of venous blood samples was not found to make a statistically significant difference in FLT kinetic analyses despite the systematically marginally higher concentration of FLT in venous plasma samples as compared to the concentration in arterial plasma samples (Visvikis *et al* 2004, Menda *et al* 2009) and venous input functions exhibited good correlation with the aortic image-derived input functions (Shields *et al* 2005). The absolute scaling of input function does not impact the stabilization curves and stabilization parameters for the same reason as the scaling of SUV. It would make difference if inter-patient correlation of FLT SUV with kinetic parameters was calculated. However, possible errors in input function scaling translates into the scaling error of K_1 , V_b and K_i parameters, which affect the correlation of stabilization parameters with tumour-averaged kinetic parameters.

Clinical implications

High correlation between the early SUV and V_b kinetic parameter (and K_1 in limited number of cases) could potentially be exploited for imaging V_b kinetic parameters with early SUV; the approach has already been proposed for the FDG PET (Strauss *et al* 2003). However, the correlation between the SUV and V_b kinetic parameter is high only in a very short time period that is case dependant. Therefore, it would be very difficult to get the quantitatively accurate V_b kinetic parameters from early SUV images. On the other hand, qualitative estimation of V_b parameters is indeed possible with the visual selection of appropriate time frame – the procedure that is often applied for vessel segmentation for input function extraction from PET images.

The FLT influx parameter is believed to be determined by the cellular proliferation rate. However, SUV is often used instead of the FLT influx parameter because of its simplicity. SUV usage assumes stable relation between the FLT influx parameter and SUV. This study has demonstrated that this relation generally exists, with possible exceptions. Observed high correlation of the TTS^{-1} for K_i stabilization curve with the average k_3 and K_i parameters implies that the tumours with higher uptake stabilize faster. However, the correlation between the K_i and SUV dropped at late time post-injection, with the drop being proportional to the average k_4 parameter. Therefore, the SUV with the too long uptake period may be of limited validity, as the too early SUV is not valid estimate of FLT influx rate. Therefore, the recommendations for FLT PET imaging interval should not be limited to minimal uptake period, but should also specify the maximal uptake period.

Experimental data shows that the FLT PET images can be quantified with the SUV only for the cases with high FLT avidity. For those cases the FLT uptake stabilizes soon (i.e. in

approximately 30 min) and converges to the FLT influx parameter. At later times the FLT uptake is impaired because of the dephosphorylation of the FLT-phosphate, so appropriate imaging period might be in the interval from 30 min to 50 min post-injection – this is the time period when all patients that featured high SUV- K_i correlation had this correlation within 0.5% of its maximum. The tumours with low FLT avidity often (but not always) stabilize slowly and their stationary FLT uptake is often poorly correlated with the FLT influx parameter. Those cases require kinetic analysis for FLT influx quantification. Decision of whether the FLT PET images will be quantified with the SUV or kinetic analysis may be based on the expected range of the FLT influx parameters. However, the FLT avidity may be hard to predict in advance and outliers are always possible. If the SUV is selected as a quantification technique for any reason, the low SUV's (below 2 for this particular dataset) should be treated with great caution, although low SUV does not necessary mean low correlation between the FLT SUV and FLT influx parameter.

Because these recommendations are based on clinical data from canine patients with two histological tumour types, quantitative results may be different for human patients. Differences would arise because of different kinetics of FLT tissue uptake and different shapes of input functions due to FLT metabolism in liver that is not present in canines and different kinetics of the FLT excretion from the blood pool. However, mechanisms of FLT tissue uptake are the same, so we can expect qualitatively similar results and recommendations.

Conclusion

This study investigated heterogeneity in FLT tissue uptake stabilization. It found that the FLT SUV is generally correlated to the V_b kinetic parameter (the vasculature fraction) immediately post-injection. At the same time or slightly later (a few minutes later), maximum in correlation with the K_j kinetic parameter – the perfusion/permeability parameter is reached, although this maximum is often lower than the SUV- V_b correlation. At later times (30+ minutes) post-injection the FLT SUV usually shows high correlation to the K_i kinetic parameter – the FLT influx parameter – that can decrease at late time post-injection. The SUV- K_i relation is often violated for regions with low FLT avidity; in our case for SUV_{30–50min} values below 2.

Based on these findings we can recommend that the FLT PET imaging protocol define the minimal and maximal FLT uptake period. For the canine patients with nasal sarcomas and carcinomas the uptake period recommendations would be 30–50 min, but for human patients this recommendation may be different. Additionally, we recommend that kinetic analysis is used when low FLT avidity is expected, and low SUV's should be treated with great caution.

Acknowledgments

This work was funded by NIH grant 1R01CA136927 and Slovenian Research Agency (ARRS). The Centre of Excellence for Biosensors, Instrumentation and Process Control is an operation financed by the European Union, European Regional Development Fund and Republic of Slovenia, Ministry of Higher Education, Science and Technology. Authors would like to acknowledge that this study relied on the tumour segmentation that was

performed by Lyndsay N. Kubicek, Michael Deveau, and Lisa Forrest, and metabolite analysis of blood samples that was performed by Matthew D. La Fontaine and Peter Scully.

References

- Barthel H, Cleij MC, Collingridge DR, Hutchinson OC, Osman S, He Q, Luthra SK, Brady F, Price PM, Aboagye EO. 3'-deoxy-3'-[18F]fluorothymidine as a new marker for monitoring tumor response to antiproliferative therapy in vivo with positron emission tomography. *Cancer Res.* 2003; 63(13):3791–3798. [PubMed: 12839975]
- Boellaard R, et al. FDG PET and PET/CT: EANM procedure guidelines for tumour PET imaging: version 1.0. *Eur J Nucl Med Mol Imaging.* 2010; 37(1):181–200. [PubMed: 19915839]
- Contractor KB, et al. Biological basis of [(1)(1)C]choline-positron emission tomography in patients with breast cancer: comparison with [(1)(8)F]fluorothymidine positron emission tomography. *Nucl Med Commun.* 2011; 32(11):997–1004. [PubMed: 21862943]
- de Langen AJ, Klabbers B, Lubberink M, Boellaard R, Spreeuwenberg MD, Slotman BJ, de Bree R, Smit EF, Hoekstra OS, Lammertsma AA. Reproducibility of quantitative 18F-3'-deoxy-3'-fluorothymidine measurements using positron emission tomography. *Eur J Nucl Med Mol Imaging.* 2009; 36(3):389–395. [PubMed: 18931838]
- Delbeke D, et al. Procedure guideline for tumor imaging with 18F-FDG PET/CT 1.0. *J Nucl Med.* 2006; 47(5):885–895. [PubMed: 16644760]
- Dittmann H, Dohmen BM, Paulsen F, Eichhorn K, Eschmann SM, Horger M, Wehrmann M, Machulla HJ, Bares R. [18F]FLT PET for diagnosis and staging of thoracic tumours. *Eur J Nucl Med Mol Imaging.* 2003; 30(10):1407–1412. [PubMed: 12898201]
- Huang SC. Anatomy of SUV. *Standardized uptake value Nucl Med Biol.* 2000; 27(7):643–646.
- Jeraj R, Liu G, Simoncic U, Vanderhoek M, Perlman S, Alberti DB, Harrison MR, Wilding G. Concurrent assessment of vasculature and proliferative pharmacodynamics in patients treated with VEGFR TKI. *Journal of Clinical Oncology (Meeting abstracts).* 2010; 28(15_suppl):3050.
- Keyes JW Jr. SUV: standard uptake or silly useless value? *J Nucl Med.* 1995; 36(10):1836–1839. [PubMed: 7562051]
- Kim SJ, Lee JS, Im KC, Kim SY, Park SA, Lee SJ, Oh SJ, Lee DS, Moon DH. Kinetic modeling of 3'-deoxy-3'-18F-fluorothymidine for quantitative cell proliferation imaging in subcutaneous tumor models in mice. *J Nucl Med.* 2008; 49(12):2057–2066. [PubMed: 18997037]
- Mankoff DA, Shields AF, Graham MM, Link JM, Eary JF, Krohn KA. Kinetic analysis of 2-[carbon-11]thymidine PET imaging studies: compartmental model and mathematical analysis. *J Nucl Med.* 1998; 39(6):1043–1055. [PubMed: 9627342]
- Menda Y, Boles Ponto LL, Dornfeld KJ, Tewson TJ, Watkins GL, Schultz MK, Sunderland JJ, Graham MM, Buatti JM. Kinetic analysis of 3'-deoxy-3'-(18)F-fluorothymidine ((18)F-FLT) in head and neck cancer patients before and early after initiation of chemoradiation therapy. *J Nucl Med.* 2009; 50(7):1028–1035. [PubMed: 19525472]
- Muzi M, Mankoff DA, Grierson JR, Wells JM, Vesselle H, Krohn KA. Kinetic modeling of 3'-deoxy-3'-fluorothymidine in somatic tumors: mathematical studies. *J Nucl Med.* 2005a; 46(2):371–380. [PubMed: 15695799]
- Muzi M, Spence AM, O'Sullivan F, Mankoff DA, Wells JM, Grierson JR, Link JM, Krohn KA. Kinetic analysis of 3'-deoxy-3'-18F-fluorothymidine in patients with gliomas. *J Nucl Med.* 2006; 47(10):1612–1621. [PubMed: 17015896]
- Muzi M, Vesselle H, Grierson JR, Mankoff DA, Schmidt RA, Peterson L, Wells JM, Krohn KA. Kinetic analysis of 3'-deoxy-3'-fluorothymidine PET studies: validation studies in patients with lung cancer. *J Nucl Med.* 2005b; 46(2):274–282. [PubMed: 15695787]
- Price SJ, et al. Imaging regional variation of cellular proliferation in gliomas using 3'-deoxy-3'-[18F]fluorothymidine positron-emission tomography: an image-guided biopsy study. *Clin Radiol.* 2009; 64(1):52–63. [PubMed: 19070698]
- Schiepers C, Dahlbom M, Chen W, Cloughesy T, Czernin J, Phelps ME, Huang SC. Kinetics of 3'-deoxy-3'-18F-fluorothymidine during treatment monitoring of recurrent high-grade glioma. *J Nucl Med.* 2010; 51(5):720–727. [PubMed: 20395318]

- Shankar LK, et al. Consensus recommendations for the use of 18F-FDG PET as an indicator of therapeutic response in patients in National Cancer Institute Trials. *J Nucl Med*. 2006; 47(6):1059–1066. [PubMed: 16741317]
- Shields AF, Briston DA, Chandupatla S, Douglas KA, Lawhorn-Crews J, Collins JM, Mangner TJ, Heilbrun LK, Muzik O. A simplified analysis of [18F]3'-deoxy-3'-fluorothymidine metabolism and retention. *Eur J Nucl Med Mol Imaging*. 2005; 32(11):1269–1275. [PubMed: 15991018]
- Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanoff JC, Lawhorn-Crews JM, Obradovich JE, Muzik O, Mangner TJ. Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. *Nat Med*. 1998; 4(11):1334–1336. [PubMed: 9809561]
- Shinomiya A, et al. Evaluation of 3'-deoxy-3'-[18F]-fluorothymidine (18F-FLT) kinetics correlated with thymidine kinase-1 expression and cell proliferation in newly diagnosed gliomas. *Eur J Nucl Med Mol Imaging*. 2013; 40(2):175–185. [PubMed: 23229746]
- Simoncic U, Jeraj R. WE-D-L100J 05: CuATSM and FLT PET Images Stabilization Assessment. *Medical Physics*. 2007; 34(6):2596–2596.
- Simoncic U, Jeraj R. Cumulative input function method for linear compartmental models and spectral analysis in PET. *J Cereb Blood Flow Metab*. 2011; 31(2):750–756. [PubMed: 20808319]
- Smyczek-Gargya B, Fersis N, Dittmann H, Vogel U, Reischl G, Machulla HJ, Wallwiener D, Bares R, Dohmen BM. PET with [18F]fluorothymidine for imaging of primary breast cancer: a pilot study. *Eur J Nucl Med Mol Imaging*. 2004; 31(5):720–724. [PubMed: 14991243]
- Strauss LG, Dimitrakopoulou-Strauss A, Haberkorn U. Shortened PET data acquisition protocol for the quantification of 18F-FDG kinetics. *J Nucl Med*. 2003; 44(12):1933–1939. [PubMed: 14660719]
- Thie JA. Understanding the standardized uptake value, its methods, and implications for usage. *J Nucl Med*. 2004; 45(9):1431–1434. [PubMed: 15347707]
- Ullrich R, Backes H, Li H, Kracht L, Miletic H, Kesper K, Neumaier B, Heiss WD, Wienhard K, Jacobs AH. Glioma proliferation as assessed by 3'-fluoro-3'-deoxy-L-thymidine positron emission tomography in patients with newly diagnosed high-grade glioma. *Clin Cancer Res*. 2008; 14(7):2049–2055. [PubMed: 18381944]
- Vesselle H, Grierson J, Muzi M, Pugsley JM, Schmidt RA, Rabinowitz P, Peterson LM, Vallieres E, Wood DE. In vivo validation of 3'-deoxy-3'-[(18)F]fluorothymidine ([18F]FLT) as a proliferation imaging tracer in humans: correlation of [18F]FLT uptake by positron emission tomography with Ki-67 immunohistochemistry and flow cytometry in human lung tumors. *Clin Cancer Res*. 2002; 8(11):3315–3323. [PubMed: 12429617]
- Visvikis D, Francis D, Mulligan R, Costa DC, Croasdale I, Luthra SK, Taylor I, Ell PJ. Comparison of methodologies for the in vivo assessment of 18FLT utilisation in colorectal cancer. *Eur J Nucl Med Mol Imaging*. 2004; 31(2):169–178. [PubMed: 15129698]
- Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: Evolving Considerations for PET response criteria in solid tumors. *J Nucl Med*. 2009; 50(Suppl 1):122S–150S. [PubMed: 19403881]
- Young H, Baum R, Cremerius U, Herholz K, Hoekstra O, Lammertsma AA, Pruim J, Price P. Measurement of clinical and subclinical tumour response using [18F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. European Organization for Research and Treatment of Cancer (EORTC) PET Study Group. *Eur J Cancer*. 1999; 35(13):1773–1782. [PubMed: 10673991]

Appendix

The solution of the FLT model that is defined in Eq. 1 and Eq. 2 is given in Eq. 3, with $IRF(t)$ being the impulse response function.

$$\begin{aligned}
C_i(t) &= IRF(K_1, k_2, k_3, k_4, t) \otimes I_p(t) \\
IRF(t) &= \frac{K_1}{\alpha_2 - \alpha_1} \left((k_3 + k_4 - \alpha_1) e^{-\alpha_1 t} + (\alpha_2 - k_3 - k_4) e^{-\alpha_2 t} \right) \\
\alpha_1 &= \frac{1}{2} \left(k_2 + k_3 + k_4 - \sqrt{(k_2 + k_3 + k_4)^2 - 4k_2 k_4} \right) \\
\alpha_2 &= \frac{1}{2} \left(k_2 + k_3 + k_4 + \sqrt{(k_2 + k_3 + k_4)^2 - 4k_2 k_4} \right)
\end{aligned} \quad (3)$$

The k_4 parameter is usually small, relative to the parameter k_2 . Therefore we can derive the $IRF(t)$ into power series of k_4 around $k_4=0$. When deriving the $IRF(t)$ to the first order we get:

$$IRF(t) = \frac{K_1}{k_2 + k_3} (k_3 + k_2 e^{-(k_2 + k_3)t}) - \frac{K_1 k_2 k_3 k_4}{(k_2 + k_3)^3} \left((2 + (k_2 + k_3)t) e^{-(k_2 + k_3)t} - 2 + (k_2 + k_3)t \right) \quad (4)$$

The first term in Eq. 4 is a known solution for a two-tissue compartment three-parameter model, while the second term is a correction for small but non-zero k_4 .

As evident from the Eqs. 2 and 3, the exact shape of $tTAC(t)$ depends on the shape of the input function $I_p(t)$. While each input function is unique and generally requires numerical evaluation of the Eq. 3, two hypothetical cases, the single impulse (delta function) and a constant blood plasma activity, can be evaluated analytically. For the impulse input function the compartmental model output $C_i(t)$ is by definition equal to the $IRF(t)$. For the constant input function with the AUC normalized to one, the compartmental model output $C_i(t)$ is:

$$\begin{aligned}
C_i(t) &= \frac{1}{T} \int_0^t IRF(K_1, k_2, k_3, k_4, \tau) d\tau \\
C_i(t) &= \frac{K_1 k_3}{k_2 + k_3} \frac{1}{T} \left(t + \frac{k_2}{k_3} \frac{1 - e^{-(k_2 + k_3)t}}{k_2 + k_3} - \frac{K_1 k_2 k_3 k_4}{2(k_2 + k_3)^4} (6 - 4(k_2 + k_3)t + (k_2 + k_3)^2 t^2 - (6 + 2(k_2 + k_3)t) e^{-(k_2 + k_3)t}) \right) \quad (5)
\end{aligned}$$

Typical FLT input function consists of a sharp peak shortly after the injection and a slowly decaying plateau at later times post-injection. Therefore, it is something in-between these two hypothetical cases. For the late time post-injection, the exponentials in Eq. 4 and Eq. 5 vanishes, so the $IRF(t)$ and $C_i(t)$ becomes:

$$\begin{aligned}
IRF(t) &= \frac{K_1 k_3}{k_2 + k_3} - \frac{K_1 k_2 k_3 k_4}{(k_2 + k_3)^3} ((k_2 + k_3)t - 2) \\
C_i(t) &= \frac{K_1 k_3}{k_2 + k_3} \left(t + \frac{k_2}{k_3(k_2 + k_3)} - \frac{K_1 k_2 k_3 k_4}{2(k_2 + k_3)^4} (6 - 4(k_2 + k_3)t + (k_2 + k_3)^2 t^2) \right) \quad (6)
\end{aligned}$$

Further simplification of Eq. 6 is possible by noting that the $t \times (k_2 + k_3) \gg 1$:

$$\begin{aligned}
IRF(t \gg (k_2 + k_3)^{-1}) &= \frac{K_1 k_3}{k_2 + k_3} - \frac{K_1 k_2 k_3 k_4}{(k_2 + k_3)^2} t \\
C_i(t \gg (k_2 + k_3)^{-1}) &= \frac{K_1 k_3}{k_2 + k_3} \left(t + \frac{k_2}{k_3(k_2 + k_3)} - \frac{K_1 k_2 k_3 k_4}{2(k_2 + k_3)^2} t^2 \right) \quad (7)
\end{aligned}$$

Figure 1.

Exemplary shapes of stabilization curves for K_i parameter (a) and K_I or V_b parameters (b). Minimal and maximal correlations for the K_i stabilization curve are designated by ρ'_{min} and ρ'_{max} , respectively. Similarly, ρ_{min} and ρ_{max} designates minimal and maximal correlations for the K_I or V_b stabilization curves. Time t'_{min} is the time when the K_i stabilization curve reaches its maximum. The t'_{max} is set to be the end of scan and is fixed at 90 min. Areas over/under the curve that were used for the evaluation of stabilization parameters, are marked with color and corresponding symbol (A' , A'' or A).

Figure 2.

An exemplary illustration of how the SUVs at different time points matched the V_b parametric image, K_1 parametric image and K_i parametric image. Note that the PET images are restricted to the tumour segmentations as delineated by a veterinarian.

Figure 3.

Typical example of stabilization curves. The V_b stabilization curve has its maximum immediately after the injection and K_l stabilization curve reaches its maximum only slightly later. Correlation for K_i is low immediately post-injection. In approximately 30 min the stabilization curves for K_l and V_b fall close to zero or even below and K_i stabilization curve reaches value near unity. Almost the same values stay until the end of scan, except some slight decrease in K_i stabilization curve. This particular example had the average tumour $SUV_{30-50min}$ of 3.5.

Figure 4.

Two exceptional examples of stabilization curves. Case a) shows very slow stabilization phenomena. Case b) exhibit high correlation only in K_I and V_b stabilization curves, whereas the correlation between K_i and $SUV(t)$ is negligible over the whole imaging period. These two examples had the average tumour $SUV_{30-50min}$ of 1.2 and 1.0 for the cases a) and b), respectively.

Figure 5. Correlation plots of $TTS-I$ versus the tumour-averaged k_3 or K_i parameter. Correlation coefficient ρ and corresponding p-value are reported for the data presented with the correlation plot.

Figure 6. Correlation plots of MC versus the tumour-averaged k_3 or K_i parameter. Correlation coefficient ρ and corresponding p-value are reported for the data presented with the correlation plot.

Figure 7. Correlation plot of CD versus the tumour-averaged k_4 parameter. Correlation coefficient ρ and corresponding p-value are reported for the data presented with the correlation plot.

Figure 8.

MC versus the average SUV. Tumours with SUV above 2 have *MC* close to unity, while others have variable *MC*s.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Means, standard deviations and ranges (evaluated over different cases) for K_i , K_I and V_b stabilization parameters TTS (time-to-stabilization), MC (maximal correlation) and CD (corrected drop from correlation maximum to the end of scan).

Stabilization curve	Stabilization parameter	mean	std	min	max
K_i	$TTS [min]$	6.9	4.1	2.5	18.2
	MC	0.72	0.23	0.11	1.00
	CD	0.021	0.023	0.00	0.077
K_I	$TTS [min]$	12.8	5.7	5.9	32.5
V_b	$TTS [min]$	11.2	5.0	1.00	24.4

Table 2

Correlations (with the corresponding p-values in brackets) between the canines' stabilization parameters (*TTS-I*, *MC* or *CD*) and their tumour-averaged kinetic parameters (K_1 , k_2 , k_3 , k_4 , V_b and K_i). Significant correlations are bolded ($p < 0.0001$ is considered significant) and high non-significant correlations are underlined.

Stabilization curve	Stabilization parameter	K_1	k_2	k_3	k_4	V_b	$K_i = \frac{K_1 k_3}{k_2 + k_3}$
K_i	<i>TTS-I</i>	0.22 (0.25)	0.12 (0.52)	<u>0.75 (<0.0001)</u>	0.054 (0.78)	0.053 (0.78)	<u>0.87 (<0.0001)</u>
	<i>MC</i>	-0.035 (0.85)	-0.035 (0.85)	<u>0.56 (0.0012)</u>	-0.28 (0.13)	0.11 (0.57)	<u>0.63 (0.0002)</u>
	<i>CD</i>	0.26 (0.16)	0.38 (0.036)	0.078 (0.68)	<u>0.77 (<0.0001)</u>	-0.14 (0.46)	-0.13 (0.50)
K_1	<i>TTS-I</i>	0.20 (0.28)	0.16 (0.39)	0.25 (0.19)	-0.34 (0.064)	0.41 (0.023)	0.31 (0.10)
V_b	<i>TTS-I</i>	0.047 (0.80)	-0.0767 (0.69)	-0.26 (0.16)	-0.18 (0.33)	0.17 (0.38)	-0.18 (0.34)

Table 3

Tumour-averaged $SUV_{30-50min}$, MC and time ranges for the $SUV(t)-K_i$ correlation above certain thresholds; reported for the group of scans with high $SUV-K_i$ correlation. The EOS stands for the *End Of Scan* being at 90 min, and indicate that $SUV-K_i$ correlation would fall below the threshold after the end of scan.

Patient – therapy	$SUV_{30-50min}$	MC	$\rho > 0.9$		$\rho > 0.98 \times MC$		$\rho > 0.995 \times MC$	
			t_{min} [min]	t_{max} [min]	t_{min} [min]	t_{max} [min]	t_{min} [min]	t_{max} [min]
PT4 – pre-therapy	3.0	0.97	7.5	EOS	13.6	EOS	19.9	54.3
PT5 – pre-therapy	4.0	0.99	5.2	EOS	17.2	EOS	25.8	63.4
PT7 – pre-therapy	3.5	0.98	6.6	EOS	14.0	EOS	24.6	EOS
PT8 – pre-therapy	4.7	1.00	3.4	EOS	10.2	EOS	18.2	68.4
PT10 – pre-therapy	1.6	0.97	5.9	EOS	11.5	EOS	16.8	55.0
PT4 – mid-therapy	2.8	0.95	10.0	EOS	14.7	EOS	23.3	75.7
PT7 – mid-therapy	1.4	0.97	11.1	EOS	17.4	68.7	21.9	51.3
PT8 – mid-therapy	3.7	0.99	4.8	EOS	12.8	EOS	23.6	68.0
PT12 – mid-therapy	1.0	0.94	19.0	EOS	22.6	60.9	28.8	49.7

Table 4

Time-dependant fraction of FLT metabolites in blood plasma

Patient – therapy	Metabolite fraction at various times post-injection [%]			
	5 min	15 min	30 min	90 min
PT1 – pre-therapy	0.7	0.5	1.8	4.4
PT1 – mid-therapy	2.9	1.2	2.6	2.6
PT2 – pre-therapy	1.6	1.6	1.3	2.5
PT2 – mid-therapy	2.8	0.8	3.1	2.6
Average	2.0	1.0	2.2	3.0