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Accounting for pharmacokinetic differences in dual-tracer receptor density imaging

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Abstract

Dual-tracer molecular imaging is a powerful approach to quantify receptor expression in a wide range of tissues by using an untargeted tracer to account for any nonspecific uptake of a molecular-targeted tracer. This approach has previously required the pharmacokinetics of the receptor-targeted and untargeted tracers to be identical, requiring careful selection of an ideal untargeted tracer for any given targeted tracer. In this study, methodology capable of correcting for tracer differences in arterial input functions, as well as binding-independent delivery and retention, is derived and evaluated in a mouse U251 glioma xenograft model using an Affibody tracer targeted to epidermal growth factor receptor (EGFR), a cell membrane receptor overexpressed in many cancers. Simulations demonstrated that blood, and to a lesser extent vascular-permeability, pharmacokinetic differences between targeted and untargeted tracers could be quantified by deconvolving the uptakes of the two tracers in a region of interest devoid of targeted tracer binding, and therefore corrected for, by convolving the uptake of the untargeted tracer in all regions of interest by the product of the deconvolution. Using fluorescently labelled, EGFR-targeted and untargeted Affibodies (known to have different blood clearance rates), the average tumor concentration of EGFR in 4 mice was estimated using dual-tracer kinetic modelling to be 3.9 ± 2.4 nM compared to an expected concentration of 2.0 ± 0.4 nM. However, with deconvolution correction a more equivalent EGFR concentration of 2.0 ± 0.4 nM was measured.

Keywords

fluorescence imaging; tracer kinetics; cancer; xenograft mouse model; epidermal growth factor receptor; affibodies

1. Introduction

Dual-tracer receptor concentration imaging (DT-RCI) is an emerging molecular imaging approach that has the potential to quantify biomolecule concentrations in a wide range of tissues *in vivo* (Tichauer *et al.*, 2012b). The approach involves imaging the temporal uptake of an untargeted tracer concurrently with the uptake of a molecular targeted tracer (Liu *et al.*, 2009; Pogue *et al.*, 2010). The uptake of the untargeted tracer can then essentially be used as a surrogate of the nonspecific component of targeted tracer uptake to better identify binding related uptake and retention (Wang *et al.*, 2012). By using reference tissue mathematical models (Lammertsma and Hume, 1996; Logan *et al.*, 1996) from the neurotransmitter positron emission tomography community, the uptake of the targeted and untargeted tracers can be used to quantify binding potential – a parameter proportional to the concentration of the biomolecule targeted by the tracer (Innis *et al.*, 2007) – for tissues of interest having no suitable reference tissue, such as in cancer imaging (Tichauer *et al.*, 2012a). One major limitation of the DT-RCI method is that it requires the arterial input functions (*i.e.*, the time-varying concentration of tracer in blood) and the ratio of tissue delivery and retention rates (*i.e.*, K_1/k_2) to be the same for the targeted and untargeted tracers. This stipulation can limit the number of untargeted tracers suitable for any given targeted tracer considering there is an inverse relationship between the molecular weight of a tracer and its vascular permeability (de Lussanet *et al.*, 2005). It is also likely that other more subtle chemical properties of a tracer will affect its clearance rate from the blood (Olafsen and Wu, 2010; Choi *et al.*, 2013). Consequently, it can be tedious and time-consuming to identify ideal targeted-untargeted tracer pairs for every new application. DT-RCI would be more adaptable if a single untargeted tracer could work for many targeted tracers.

Differences in tracer delivery and retention (K_1 and k_2) are governed by vascular permeability, which in turn has been found to be proportional to a tracer's size, charge, and lipophilicity (de Lussanet *et al.*, 2005; Yaehne *et al.*, 2013; Yuan *et al.*, 1995). In general, this implies that the similarity between K_1 and k_2 can be controlled by selecting targeted and untargeted tracers with similar sizes and charges. On the other hand, differences in tracer plasma pharmacokinetic/clearance rates can be based on much more subtle molecular characteristics (Choi *et al.*, 2013; Olafsen and Wu, 2010). It is for these reasons that in this study a method is described that expands the selection of tracer pairs by correcting for differences in blood clearance rates between targeted and untargeted tracers. Errors associated with tracer differences in tissue delivery and retention rates are also investigated. The approach entails calculating a function relating the uptakes of the two tracers in a region of interest devoid of targeted tracer via a deconvolution algorithm (Diop and St Lawrence, 2012). The “deconvolved” curve is then re-convolved with all untargeted tracer uptakes in a temporal imaging stack to correct for differences in tracer pharmacokinetics. The utility of this approach is verified experimentally in a mouse tumor xenograft model through fluorescence DT-RCI of epidermal growth factor receptor (EGFR), a cell surface receptor overexpressed in many forms of cancer (Nicholson *et al.*, 2001); and theoretically through mathematical analysis of targeted and untargeted tracer pharmacokinetic differences using

compartment model solutions of targeted and untargeted tracer uptake in tissue (Kety, 1951; Lammertsma *et al.*, 1996).

2. Theory

2.1 Propagation of arterial input function discordance between targeted and untargeted tracer

In DT-RCI, the uptake kinetics of the targeted tracer are generally represented by a two-tissue compartment model [Fig. 1(c)] (Mintun *et al.*, 1984), while those of the untargeted tracer (used to account for non-specific uptake of the targeted tracer) are represented by a one-tissue compartment model [Fig 1(d)] (Kety, 1951). A system of differential equations can be used to represent the targeted tracer two-tissue compartment kinetic model mathematically as follows:

$$\begin{aligned}\frac{dC_{f,T}(t)}{dt} &= K_1 C_{a,T}(t) - (k_2 + k_3) C_{f,T}(t) + k_4 C_{b,T}(t) \text{ and} \\ \frac{dC_{b,T}(t)}{dt} &= k_3 C_{f,T}(t) - k_4 C_{b,T}(t),\end{aligned}\quad (1)$$

where $C_{a,T}$, $C_{f,T}$, and $C_{b,T}$ represent the concentrations of targeted tracer in the arterial plasma, in the extravascular space (unbound or “freely associated”), and in the extravascular space and bound to the targeted cell surface receptor, respectively; K_1 and k_2 are the rate constants governing transport of the targeted tracer between the blood and extravascular space; and k_3 and k_4 are the rate constants governing association and dissociation of targeted tracer binding. A similar differential equation can be used to represent the untargeted tracer one-compartment kinetic model:

$$\frac{dC_{f,U}(t)}{dt} = K_{1,U} C_{a,U}(t) - k_{2,U} C_{f,U}(t), \quad (2)$$

where $C_{a,U}$ and $C_{f,U}$ represent the concentrations of the untargeted tracer in the arterial plasma and freely associated in the extravascular space, respectively; and $K_{1,U}$ and $k_{2,U}$ are the rate constants governing transport of the untargeted tracer between the blood and extravascular space. To this point, all DT-RCI applications have assumed that the arterial input functions of the targeted and untargeted tracers were identical. Therefore, the differential equations in Eq. (1) and (2) could be solved to represent the measured uptake of the targeted tracer in any region of interest, ROI_T , as a function of the measured uptake of the untargeted tracer in the same region of interest, ROI_U , using the “simplified reference tissue model” approach (Lammertsma and Hume, 1996) as follows (Tichauer *et al.*, 2012b):

$$ROI_T(t) = R_1 ROI_U(t) + k_2 \left[1 - \frac{R_1}{1+BP} \right] ROI_U(t) * e^{\frac{k_2}{1+BP}t}, \quad (3)$$

where $R_1 = K_1/K_{1,U}$, $BP = k_3/k_4$, and $*$ represents the convolution operator. The “binding potential”, BP , is typically the salient parameter of interest as it is proportional to the concentration of targeted receptors available for tracer binding in a region of interest (Innis *et al.*, 2007). The simplified reference tissue model approach applied to DT-RCI makes four main assumptions in an effort to reduce the number of distinct physiological parameters in

data-fitting and thereby improve the numerical accuracy of parameter estimates (Gunn *et al.*, 1997):

- 1) K_1/k_2 is equivalent to $K_{1,U}/k_{2,U}$ (see Discussion for applicability to dual-tracer imaging)
- 2) The free and bound concentrations of the tracer in the tissue [C_f & C_b from Fig. 1(c), respectively] are in an instantaneous equilibrium [*i.e.*, $C_{b,T}(t)/C_{f,T}(t)$ is equal to a constant, k_3/k_4 , at all t]
- 3) Tracer signal from the blood volume is negligible
- 4) The arterial input functions of the targeted and untargeted tracers are sufficiently equivalent (Tichauer *et al.*, 2012b)

In this study, we examined Eq. (3) if assumption #4 cannot be made. If the arterial input functions of a targeted tracer, $C_{a,T}(t)$, and untargeted tracer, $C_{a,U}(t)$, were to differ as a function of time, t , an expression can be derived to relate the two functions as follows:

$$C_{a,T}(t) = C_{a,U}(t) * g(t), \quad (4)$$

where $g(t)$ is a function that converts $C_{a,U}(t)$ to $C_{a,T}(t)$. By substituting Eq. (4) into the first expression in Eq. (1) and resolving for Eq (3), Eq. (3) is modified as follows:

$$ROI_T(t) = R_1 ROI_U(t) * g(t) + k_2 \left[1 - \frac{R_1}{1+BP} \right] ROI_U(t) * g(t) * e^{-\frac{k_2}{1+BP}t}. \quad (5)$$

Therefore, if $g(t)$ were known, BP could be estimated from Eq. (5) through non-linear fitting, even though the arterial input functions of the two tracers were different.

$g(t)$ can be determined from Eq. (4) if the arterial input functions of the targeted and untargeted tracers were measured; however, this would require invasive blood sampling. A more flexible approach can be realized by comparing the uptake of the targeted tracer to the untargeted tracer in a tissue devoid of targeted binding (*i.e.*, where $BP = 0$). With $BP = 0$, and assuming $R_1 \approx 1$ (*i.e.*, that $K_1 \approx K_{1,U}$), Eq. (5) simplifies as follows:

$$ROI_T(t) = ROI_U(t) * g(t), \quad (6)$$

for $BP=0$ and $K_1=K_{1,U}$

In this receptor-free tissue, $g(t)$ can be determined by deconvolving $ROI_T(t)$ by $ROI_U(t)$. Deconvolution is typically an unstable procedure so we adopted the approach developed by Diop and St. Lawrence, which is based on general singular value decomposition and Tikhonov regularization (Diop and St Lawrence, 2012).

3. Methods and Results

3.1 Animal experiment

To assess the deconvolution approach *in vivo*, ten female nude mice (Charles River Laboratories, Wilmington, MA) were inoculated subcutaneously on the right thigh with 10^5

cells of human U251 glioma line, which is known to express a moderate level of epidermal growth factor receptor (EGFR) (Tichauer *et al.*, 2012b). When the tumors reached a diameter of 3 mm, the skin was removed from the tumors of four mice to measure tracer uptake in the tumor, and the carotids were exposed in the remaining 6 mice for a direct measure of the arterial input functions of the tracers (Elliott *et al.*, 2014). All mice were injected with an anti-EGFR (targeted) affibody molecule and a negative control (untargeted) affibody (Affibody Ab, Solna, Sweden), which were labelled with the fluorophores, IRDye-800CW and IRDye-680RD (LI-COR Biosciences, Lincoln, NE), respectively. A mixed solution containing 0.1 nanomoles of each tracer was injected into a tail vein of each mouse. In the four mice with exposed tumors, the uptake of these targeted and untargeted tracers was imaged in the tumor (“tissue of interest”) and the leg muscle (devoid of EGFR; “receptor-free tissue”) on a two-wavelength planar fluorescence imaging system (Odyssey, LI-COR Biosciences) at 1 min intervals for 40 min post-injection. In the six mice with exposed carotids, the arterial input functions of both tracers were imaged directly from the exposed vessels.

Targeted and untargeted tracer uptakes in the receptor-free tissue of all four tumor-exposed mice are presented in Fig. 2(b), after an early uptake normalization to account for regional differences in detection efficiency of the two tracers (Tichauer *et al.*, 2013). Despite no expected EGFR binding in the muscle, the two tracers were found to have considerably different uptake kinetics in all animals, reflecting a difference in arterial input functions [supported by the measured tracer arterial input functions from the six carotid-exposed mice – Fig. 2(a)], vascular permeability, and/or nonspecific binding between the two tracers. Interestingly though, when each targeted-untargeted tracer pair was deconvolved, the resulting $g(t)$ s were very similar in all animals [Fig. 2(c)]. Moreover, the deconvolved functions determined in the receptor-free tissues were not statistically different from, and appeared nearly equivalent to, the deconvolution of the arterial input functions [Black data in Fig. 2(c)] of the two tracers measured in 6 separate mice [Fig. 2(a)]. Moreover, in the 6 mice where the arterial input functions were measured, there were no statistically significant differences in k_2 values measured in receptor-free tissue between the tracers ($0.046 \pm 0.014 \text{ min}^{-1}$ for the targeted tracer compared to $0.039 \pm 0.008 \text{ min}^{-1}$ for the untargeted tracer), suggesting that the uptake differences observed were primarily related to tracer differences in blood pharmacokinetics. These $g(t)$ s were then convolved with the untargeted tracer uptakes in all pixels to attempt to correct for the inequalities in binding-independent targeted and untargeted tracer uptake. Examples of the corrected uptake curves of untargeted tracer in the muscle and tumor tissue in one of the mice are presented in Fig. 2(d). The similarity between the uptakes of the two tracers, presented in a different muscle tissue than that which was selected for the deconvolution, demonstrates that it is possible to use this approach to correct non-binding related delivery and retention differences between the two tracers. Applying the DT-RCI algorithm on a pixel-by-pixel resulted in the BP maps presented in Fig. 3, with and without using the deconvolution correction approach. For all mice, the tumor-to-background contrast-to-noise ratio was significantly higher using the correction approach (3.8 ± 2.1) compared to using the raw uptake curves (1.5 ± 0.9), with the average BP equal to 0.9 ± 0.2 and 0.0 ± 0.2 in the tumor and muscle, respectively, using the correction approach, and equal to 1.8 ± 0.9 and 1.0 ± 0.5 with no correction. With a

dissociation constant, K_D , of 2.2 ± 0.4 nM (unpublished data) for the anti-EGFR affibody, the BP can be converted to a mean EGFR concentration in the tumors of 2.0 ± 0.4 nM, which falls in line with the validated measure of EGFR concentration in these U251 tumors of 2.0 ± 0.4 nM. By comparison, the uncorrected measure of EGFR concentration of the tumors would be 3.8 ± 2.4 nM.

3.2 Arterial input function differences simulation study

To test the deconvolution approach in the presence of experimental noise, two simulation studies were carried out. In the first study, theoretical uptakes of a targeted tracer, and a “non-ideal” untargeted tracer (an untargeted tracer with a different arterial input function from the targeted tracer) were created from the two expressions in Eq. (2), respectively, in a tissue devoid of targeted molecules (“receptor-free tissue”; $BP = 0$) and in a tissue with a range of targeted molecule concentrations (“tissue of interest”). In the tissue of interest, the binding dissociation rate constant, k_4 , was assumed to be 0.04 min^{-1} , based on studies carried out on assays of monomeric binding of the anti-EGFR Affibody tracer used in the animal experiments (Friedman *et al.*, 2007). The binding association rate constant, k_3 , was assumed to be $0.008\text{--}0.24 \text{ min}^{-1}$ to match the range of EGFR concentrations expected in various tumor lines (Tichauer *et al.*, 2012b), while assuming an affinity of the anti-EGFR affibody of $K_D = 2.2$ nM (unpublished data)]. For these simulations, $C_{a,T}(t)$ and $C_{a,U}(t)$ were measured from an exposed carotid artery in 6 female nude athymic mice injected with a mixture of EGFR-targeted Affibody tracer labelled with IRDye-800CW and negative control Affibody labelled with IRDye-680RD (see Section 3.1). The average of the arterial input curves are presented in Fig. 2(a). K_1 was assumed to be 0.01 ml/min/ml in the receptor-free tissue and 0.03 ml/min/ml in the tissue of interest, and k_2 was assumed to be 0.1 min^{-1} in the receptor-free tissue and 0.08 min^{-1} in the tissue of interest, in line with prior studies (Tichauer *et al.*, 2012a). Random Gaussian noise was then added to all curves at 2% of peak-signal and the deconvolution approach was employed to determine $g(t)$ from the uptake of the targeted tracer and the non-ideal untargeted tracer in the receptor-free tissue. This $g(t)$ was then convolved with the uptake of the non-ideal targeted tracer in the tissue of interest [red curve in Fig. 4(a)], creating the green curve in Fig. 4(a), which compared well with the simulated uptake of an untargeted tracer assumed to have the same arterial input function as the targeted tracer in the tissue of interest [black dots in Fig. 4(a)]. This corrected untargeted tracer uptake was then coupled with the targeted tracer uptake [blue curve in Fig. 4(a) for $k_3 = 0.3 \text{ min}^{-1}$] in the DT-RCI fitting algorithm to estimate binding potential (Tichauer *et al.*, 2012b). Binding potential (BP) is the ratio of k_3/k_4 , defined as such because it is proportional to the concentration of targeted molecule (Innis *et al.*, 2007). Noise addition was repeated randomly, 100 times for each value of k_3 , to evaluate the impact of noise on BP estimation using the deconvolution approach. A strong correlation was observed between the BP results and the simulated value of BP [red data in Fig. 4(b)]. For comparison, BP was also estimated using the uncorrected untargeted tracer uptake in the tissue of interest as an input to the DT-RCI algorithm [blue data in Fig. 4(b)], which showed a substantial overestimation in BP as a result of the differences in arterial input functions.

3.3 Kinetic parameter differences simulation study

The second simulation study was carried out to determine if the deconvolution approach could also be used to mitigate potential differences in vascular permeability (K_1 and k_2) of the targeted and untargeted tracers in DT-RCI. Kinetic parameters from the first simulation were used with the following exceptions: the binding rate constant, k_3 , was set to 0.3 min^{-1} ($BP = 3$) in the tissue of interest; either the K_1 or the k_2 of the targeted tracer was varied by -50 to 50% in the receptor-free tissue; and $C_{a,T}(t)$ and $C_{a,U}(t)$ were taken from the mouse experiments [Fig. 2(a)] to observe the isolated effects of K_1 and k_2 differences on targeted and untargeted tracer uptakes. The absolute error in estimated BP when using the deconvolution approach to correct for K_1 and k_2 differences are presented in Fig. 4(c). Accurate BP estimation was observed with deconvolution correction for K_1 differences between tracers, since the K_1 difference will immerge as a multiplication factor in $g(t)$. Differences in k_2 led to roughly equivalent errors in BP (a 50% difference in k_2 led to a roughly 50% error in BP). From the k_2 determinations in receptor free-tissue discussed in Section 3.1, the error between the average k_2 of the two tracers was approximately 15% , leading to a potential 15% overestimation in BP .

4. Discussion and conclusion

For many cancer targeted imaging agents (e.g., antibodies or antibody fragments) it is not difficult to synthesize a second untargeted imaging agent with similar size, shape, charge, and lipophilicity (typically referred to as a negative control imaging agent), to ensure relatively equivalent K_1 and k_2 between a targeted and untargeted tracer (Yuan *et al.*, 1995; Gao *et al.*, 2004); however, blood clearance rates can depend on more subtle chemical characteristics and on targeted tracer binding (Gibaldi and Koup, 1981; Choi *et al.*, 2013; Olafsen and Wu, 2010). This study presents an approach that has the capacity to correct for pharmacokinetic differences, in particular with respect to blood clearance rates, between a targeted and an untargeted tracer, allowing a larger range of untargeted tracers to be suitable for dual-tracer experiments. More specifically, the study demonstrates that differences in the arterial input functions of a targeted and untargeted tracer can be quantified on a subject-to-subject basis within a function $g(t)$, by deconvolving the uptake of the two tracers in a tissue devoid of the targeted receptor [Eq. (6)]. While $g(t)$ can differ from subject to subject, it will not be regionally dependent for kinetic modelling time-scale studies (Mintun *et al.*, 1984): *i.e.*, it is only dependent on differences in the arterial input functions of the targeted and untargeted tracers, which themselves are assumed to have the same shape in all tissues (Lammertsma and Hume, 1996). The effect of the arterial input difference can then be corrected for in all tissues within a subject by convolving $g(t)$ with untargeted tracer uptake prior to applying DT-RCI modelling approaches to estimate targeted molecular concentrations. Moreover, simulation experiments demonstrated that this deconvolution approach could also mitigate errors associated with differences in tracer delivery and retention if K_1 and/or k_2 differences in the receptor-free tissue were proportional to K_1 and/or k_2 differences in the tissue of interest (e.g. if ratio of targeted tracer K_1 to untargeted tracer K_1 in the receptor-free tissue was equivalent to the same ratio in the tissue of interest).

As an initial *in vivo* test of the deconvolution correction approach, an EGFR-targeted/untargeted tracer pair (known from experience to demonstrate substantially different binding-independent pharmacokinetics in mice [Fig. 2(b)]) was employed to estimate the concentration of EGFR in a xenograft glioma model. Without correcting for the pharmacokinetic differences in the tracers, the EGFR concentration was overestimated by a factor of more than two, and significant EGFR expression was measured in EGFR-devoid muscle tissue (see left column of Fig. 3). However, by employing the deconvolution correction approach, measured EGFR expression in the muscle was not significantly different than zero and the average EGFR expression measured in the tumors was not significantly different than the expected level.

One limitation of this deconvolution approach is that it requires the uptake of both tracers to be measured in a tissue devoid of targeted tracer binding on a subject-by-subject basis, which may be difficult to identify depending on the molecule targeted. If a suitable receptor-free tissue cannot be identified, it may be possible to determine $g(t)$ through deconvolution of measured arterial input functions of the two tracers (as long as pharmacokinetic differences between them are primarily associated with differences in blood clearance rates). Alternatively, the results from this study suggest a less invasive approach may be possible since $g(t)$ was observed to be very similar amongst all 10 mice imaged [Fig. 2(c)], even though there were significant differences in blood and tissue uptake fluorescence curves amongst animals for the individual tracers [Fig. 2(a) and 2(c), respectively]. Therefore if $g(t)$ was known for a specific tracer in one animal, the same function could be used to correct untargeted tracer uptake in future studies without requiring further blood sampling. The average EGFR concentration measured in the 4 tumor mice in this study using a common $g(t)$ was 2.1 ± 0.5 nM compared to 2.0 ± 0.4 nM using individually determined $g(t)$ functions, with a mean error of 0.1 ± 0.3 nM (on average the error was less than 15%). In this initial study, there was no significant difference in the use of a common $g(t)$ or one that was determined on a subject-by-subject basis. Further testing will be required to explore the trade-offs of these two approaches in larger studies.

The findings of this study suggest that the deconvolution correction approach presented here could allow a larger range of appropriate untargeted tracers for any given targeted tracer, making tracer selection less cumbersome and opening the window for FDA-approved imaging agents to be used as untargeted tracers, which would facilitate clinical translation.

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References

- Choi HS, Gibbs SL, Lee JH, Kim SH, Ashitate Y, Liu F, Hyun H, Park G, Xie Y, Bae S, Henary M, Frangioni JV. Targeted zwitterionic near-infrared fluorophores for improved optical imaging. *Nature biotechnology*. 2013; 31:148–53.
- de Lussanet QG, Langereis S, Beets-Tan RG, van Genderen MH, Griffioen AW, van Engelshoven JM, Backes WH. Dynamic contrast-enhanced MR imaging kinetic parameters and molecular weight of

- dendritic contrast agents in tumor angiogenesis in mice. *Radiology*. 2005; 235:65–72. [PubMed: 15731376]
- Diop M, St Lawrence K. Deconvolution method for recovering the photon time-of-flight distribution from time-resolved measurements. *Optics letters*. 2012; 37:2358–60. [PubMed: 22739907]
- Elliott JT, Tichauer KM, Samkoe KS, Gunn JR, Sexton KJ, Pogue BW. Direct characterization of arterial input functions by fluorescence imaging of exposed carotid artery to facilitate kinetic analysis. *Molecular Imaging and Biology*. 2014 In Press.
- Friedman M, Nordberg E, Hoiden-Guthenberg I, Brismar H, Adams GP, Nilsson FY, Carlsson J, Stahl S. Phage display selection of Affibody molecules with specific binding to the extracellular domain of the epidermal growth factor receptor. *Protein engineering, design & selection : PEDS*. 2007; 20:189–99.
- Gao X, Cui Y, Levenson RM, Chung LW, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. *Nature biotechnology*. 2004; 22:969–76.
- Gibaldi M, Koup JR. Pharmacokinetic concepts - drug binding, apparent volume of distribution and clearance. *European journal of clinical pharmacology*. 1981; 20:299–305. [PubMed: 7308282]
- Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *NeuroImage*. 1997; 6:279–87. [PubMed: 9417971]
- Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang SC, Ichise M, Iida H, Ito H, Kimura Y, Koeppe RA, Knudsen GM, Knuuti J, Lammertsma AA, Laruelle M, Logan J, Maguire RP, Mintun MA, Morris ED, Parsey R, Price JC, Slifstein M, Sossi V, Suhara T, Votaw JR, Wong DF, Carson RE. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2007; 27:1533–9.
- Kety SS. The theory and applications of the exchange of inert gas at the lungs and tissues. *Pharmacological reviews*. 1951; 3:1–41. [PubMed: 14833874]
- Lammertsma AA, Bench CJ, Hume SP, Osman S, Gunn K, Brooks DJ, Frackowiak RS. Comparison of methods for analysis of clinical [¹¹C]raclopride studies. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 1996; 16:42–52.
- Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. *NeuroImage*. 1996; 4:153–8. [PubMed: 9345505]
- Liu JT, Helms MW, Mandella MJ, Crawford JM, Kino GS, Contag CH. Quantifying cell-surface biomarker expression in thick tissues with ratiometric three-dimensional microscopy. *Biophysical journal*. 2009; 96:2405–14. [PubMed: 19289065]
- Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL. Distribution volume ratios without blood sampling from graphical analysis of PET data. *J Cereb Blood Flow Metab*. 1996; 16:834–40. [PubMed: 8784228]
- Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Annals of neurology*. 1984; 15:217–27. [PubMed: 6609679]
- Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. *European journal of cancer*. 2001; 37(Suppl 4):S9–15. [PubMed: 11597399]
- Olafsen T, Wu AM. Antibody vectors for imaging. *Seminars in nuclear medicine*. 2010; 40:167–81. [PubMed: 20350626]
- Pogue BW, Samkoe KS, Hextrum S, O'Hara JA, Jermyn M, Srinivasan S, Hasan T. Imaging targeted-agent binding in vivo with two probes. *Journal of biomedical optics*. 2010; 15:030513. [PubMed: 20614996]
- Tichauer KM, Deharvengt SJ, Samkoe KS, Gunn JR, Bosenberg MW, Turk MJ, Hasan T, Stan RV, Pogue BW. Tumor Endothelial Marker Imaging in Melanomas Using Dual-Tracer Fluorescence Molecular Imaging. *Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging*. 2013

- Tichauer KM, Samkoe KS, Klubben WS, Hasan T, Pogue BW. Advantages of a dual-tracer model over reference tissue models for binding potential measurement in tumors. *Physics in medicine and biology*. 2012a; 57:6647–59. [PubMed: 23022732]
- Tichauer KM, Samkoe KS, Sexton KJ, Hextrum SK, Yang HH, Klubben WS, Gunn JR, Hasan T, Pogue BW. In vivo quantification of tumor receptor binding potential with dual-reporter molecular imaging. *Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging*. 2012b; 14:584–92. [PubMed: 22203241]
- Wang D, Chen Y, Leigh SY, Haerberle H, Contag CH, Liu JT. Microscopic Delineation of Medulloblastoma Margins in a Transgenic Mouse Model Using a Topically Applied VEGFR-1 Probe. *Translational oncology*. 2012; 5:408–14. [PubMed: 23323155]
- Yaehne K, Tekrony A, Clancy A, Gregoriou Y, Walker J, Dean K, Nguyen T, Doiron A, Rinker K, Jiang XY, Childs S, Cramb D. Nanoparticle accumulation in angiogenic tissues: towards predictable pharmacokinetics. *Small*. 2013; 9:3118–27. [PubMed: 23463664]
- Yuan F, Dellian M, Fukumura D, Leunig M, Berk DA, Torchilin VP, Jain RK. Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. *Cancer Res*. 1995; 55:3752–6. [PubMed: 7641188]

Figure 1.

A photograph of a mouse prepped for imaging is presented in (a). A greyscale image of the tumor, located as the yellow rectangle in (a), is presented in (b). Tracer kinetic compartment models are represented for a targeted tracer and an untargeted tracer in (c) and (d), respectively, under false coloured uptake maps of targeted (red scale) and untargeted (green scale) fluorescence at 40 min post tracer injection. $C_{a,T}$ and $C_{a,U}$ are the arterial input functions of the targeted and untargeted tracers, respectively; $C_{f,T}$, $C_{f,U}$, and $C_{b,T}$ represent the concentrations of targeted tracer, untargeted tracer, and bound targeted tracer, respectively, in the extravascular-extracellular or “tissue” compartment; K_1 and k_2 are the rate constants governing transport of the targeted tracer between the blood and extravascular space; $K_{1,U}$ and $k_{2,U}$ are the same thing for the untargeted tracer; k_3 and k_4 are the rate constants governing association and dissociation of targeted tracer binding.

Figure 2.

Plots of the arterial input functions for EGFR-targeted and untargeted fluorescent Affibodies measured from carotid artery fluorescence in 6 mice that were used in the simulations for targeted (blue data) and untargeted (red data) tracers are presented in (a). Lighter lines represent the respective curves from the individual mice. Darker lines represent mean $\pm$ sd of the groups. The uptake curves of the targeted tracer (solid curves) and untargeted tracer (dashed curves) in leg muscle are presented in (b) for the four mice studied where each color represents results from a separate animal. Results of deconvolution of the targeted-untargeted tracer pairs displayed in (b) are presented with corresponding colors in (c) along with the deconvolution of the arterial input functions from (a) presented in solid black. Typical “corrected” untargeted tracer uptake curves in the muscle (“Untar Ref Tissue”; dashed red curve) and tumor (“Untargeted Tumor”; solid red curve) of Mouse #2 are displayed alongside targeted tracer uptake curves in the muscle (“Tar Ref Tissue”; dashed blue curve) and tumor (“Targeted Tumor”; solid blue curve) in (d). The units of these curves are all normalized to the first measured data point.

Figure 3. Example binding potential maps produced using dual-tracer kinetic model fitting of the targeted and untargeted tracer uptake in the mouse experiments are presented for two mice without (left column) and with (right column) using the deconvolution correction approach. The white arrows locate the tumors.

Figure 4.

Typical noise-added tracer uptake curves in the tissue of interest (for $k_3 = 0.3 \text{ min}^{-1}$) are presented in (a). The targeted data are represented as the blue curve, the untargeted as red, corrected untargeted tracer as green, and “ideal” untargeted tracer (uptake of an untargeted tracer with the same arterial input function as the targeted tracer) as black dots. Estimated binding potential (BP) using dual-tracer modelling with deconvolution correction (red data) and without (blue data) are presented in (b). The black line represented the line of identity. Errors in BP from differences in K_1 (purple data) and k_2 (cyan data) between the targeted and untargeted tracers in the “receptor-free” tissue are presented in (c).