Supporting Information

Lipid nanoemulsion passive tumor accumulation dependence on tumor stage and anatomical location: A new mathematical model for *in vivo* imaging biodistribution study

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Methodology

***In Vivo* Microtomography**

Mice were also imaged with x-ray microtomography (Micro-CT) to investigate lung metastasis. Micro-CT images were obtained using the Albira System (Brucker, USA) with animals under anesthesia (ketamine and xylazine at 80 and 10 mg.kg^{-1}, respectively). Mice exams were conducted in low resolution settings (35 kV dosage, 0.2 mA voltage, 250 projections and 3 minutes of exposure), and image reconstruction was prepared using standard software option. Volume of interest of mouse lungs was selected, and the Hounsfield Unit (HU) quantification

was established for each individual lung. Higher HU density means that the lungs have denser tissues, which is a strong correlation with metastasis progression.

Mathematical Model

The following variables and constants are included in the description of Equation (1):

- C_V: average concentration of cargo in the blood vessel;

- C: average concentration of cargo in the tumor interstitial space;

- C_O: initial cargo concentration in the blood stream;

- P: effective vascular permeability;

- S: average vascular density;

- T_H: typical cargo disposition time constant;

- T_C: typical cargo delivery time constant;

The equation governing the time-dependence of the average concentration of cargo (C) in the tumor interstitial space is given by:

$$\frac{dC}{dt} = (C_V - C)/T_C \quad , \text{ (S1)}$$

with (C_V - C) scaling with the pressure difference between the blood stream and the tumor interstitial space. Here $T_C = (PS)^{-1}$ and $C_V = C_O\exp(-t/T_H)$ accounts for the disposition of the cargo in the blood stream to a third compartment of the organism (1). Notice that P and S are taken as constants for the timescale of the experiment. The general solution of the differential equation-S1 is given by:

$$C = A\exp(\alpha t) + B\exp(\beta t) \quad , \text{ (S2)}$$

where A, B, α and β in equation-S2 are constants to be determined. Taking the general solution represented by equation-S2, its first-derivative with respect to time and the expression of $C_V = C_O\exp(-t/T_H)$ back into equation-S1 and performing the required algebra one can find $\alpha=-1/T_H$, $\beta=-1/T_C$, $A= [T_H/(T_H-T_C)]C_O$, and $B=-[T_C/(T_H-T_C)]C_O$. Therefore, solution of equation-S1 above can be written as:

$$\frac{C(t)}{C_O} = \frac{1}{1-K}\left[\exp\left(-\frac{t}{T_H}\right) - K\exp\left(-\frac{t}{T_C}\right)\right] \quad , \quad (S3)$$

with $K = T_C/T_H$ and $K < 1$ for physical meaning, i.e. $T_C < T_H$. The cargo disposition time constants fitted for the data collected from the metastasis (data presented in Figures 8B-8E) are equal to $T_H = 33.2 \pm 0.1$ weeks for NEDiR and $T_H = 34.4 \pm 0.3$ weeks for DiR. Likewise, the fitted values for the cargo disposition time constants found for the data collected from the primary tumor are $T_H = 2.42 \pm 0.01$ weeks (NEDiR) and $T_H = 1.30 \pm 0.01$ weeks (DiR).

Table S1. Primary tumor biodistribution. Values represent average and standard deviation.

Week	DiR	NEDiR
Absolute Values		
1	2.3×10^8	7.02×10^7
2	2.7×10^8	1.07×10^8
3	1.2×10^8	1.41×10^8
4	2.12×10^8	2.37×10^8
Statistical Data		
r^2	0.1721	0.9293
1/slope	-4.902×10^{-8}	1.871×10^{-8}
p value (*significance $p<0.05$)	0.5851	0.0360*

Supplementary Figure Captions

Figure S1: Lung *in vivo* micro computed tomography. Panel A represents the transversal section of experimental mice with nodular metastatic lesions (*) and the normal black alveolar space (#). In panel B the voxel density displacement to the right comparing week 1 and week 4 is presented. In panel C the macroscopic aspect of the metastatic lungs is presented (black arrow indicates the metastatic lung nodules).

Figure S2: Comparison of the primary and metastatic percentage growth in panel A. In panel B the ratio between these two data is presented. The dotted lines (red, blue, and black) are guide to the eye and represent the normal Gaussian distribution of the data.

Figure S3: Ratio between left and right DiR fluorescence. The dotted lines (red and blue) are guide to the eye and represent the linear correlation between the data.

Figure S4: Typical DiR fluorescence signals detected in an experimental mouse. Yellow circle highlights the NEDiR fluorescent signal in the primary tumor.

Bibliographic References:

1. Lacava LM, Lacava ZG, Da Silva MF, Silva O, Chaves SB, Azevedo RB, et al. Magnetic resonance of a dextran-coated magnetic fluid intravenously administered in mice. Biophys J. 2001;80(5):2483-6.