

# Development of a Physiologically-Based Pharmacokinetic Model for tumor disposition of Nanoparticles Based-Therapeutics

Marta Rodríguez Jiménez<sup>1</sup>, Alejandro Serrano<sup>1,2</sup>, Ainara Salgado<sup>1</sup>, Noelia Casares<sup>2,4</sup>, Teresa Lozano<sup>2,4</sup>, Juan José Lasarte<sup>2,4</sup>, Sara Zalba<sup>1,2</sup>, Iñaki F Trocóniz<sup>1,2,3</sup>, María J. Garrido<sup>1,2</sup>

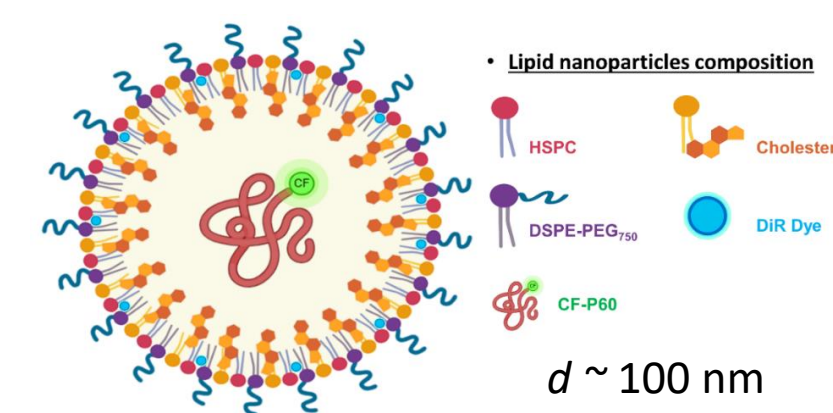
(1) Department of Pharmaceutical Sciences, School of Pharmacy and Nutrition, University of Navarra, Pamplona, Spain. (2) Navarra Institute for Health Research (IdiSNA), Pamplona, Spain. (3) Institute of Data Science and Artificial Intelligence, DATAI, University of Navarra, Pamplona, Spain. (4) Program of Immunology and Immunotherapy, CIMA, Pamplona, Spain.

## INTRODUCTION

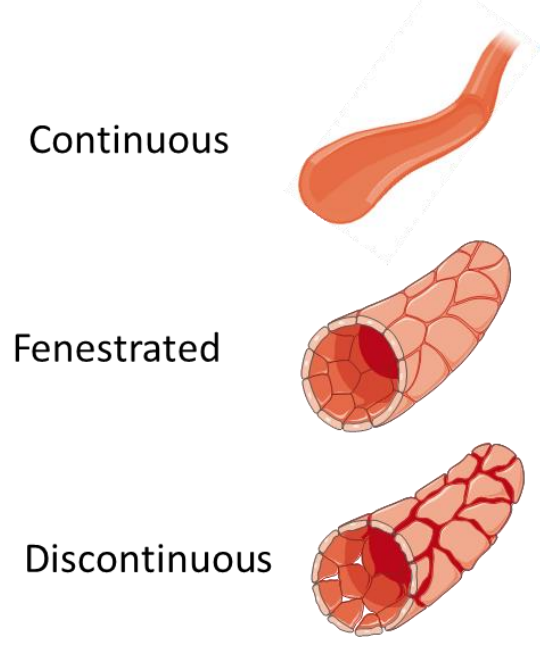
The objective of this study is to develop a **Physiologically-Based Pharmacokinetic (PBPK)** model for **lipid nanoparticles (NP) encapsulating P60 peptide** following a **single intravenous administration** in MC38 tumor-bearing mice. P60, a short-chain peptide (sequence RAFQAFRKMWPFFAM), is a FOXP3 inhibitor that modulates regulatory T cells (Tregs). In preclinical models, P60 has demonstrated efficacy both as monotherapy and in combination, hampering tumor progression. However, its short half life in circulation limit its clinical translation, requiring high and daily doses to achieve tumor regression response. To overcome this limitation, P60 has been encapsulated into lipid NP to increase its systemic stability and improve tumor disposition.

In the last decade, PBPK model development has increased for traditional pharmaceutical formulations. However, its application in new delivery systems as NP is still scarce. Since they are large and polar molecules, several factors should be considered:

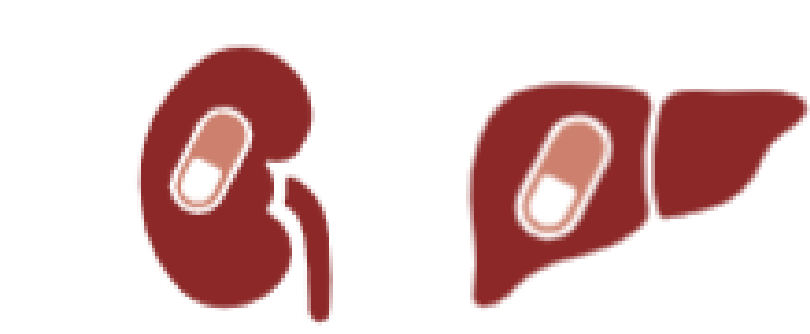
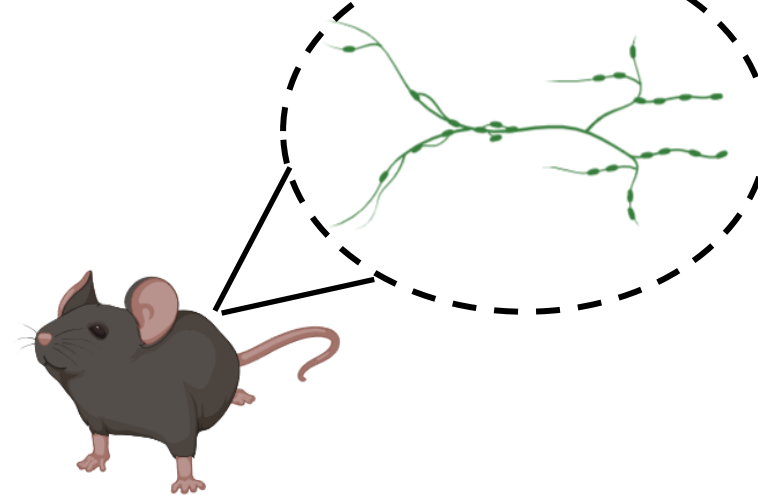
(i) permeability-limited models are more suitable for characterizing their distribution



(ii) nanoparticles extravasate through the pores in the endothelium membrane of blood vessels, leading to a size-dependent and heterogeneous distribution

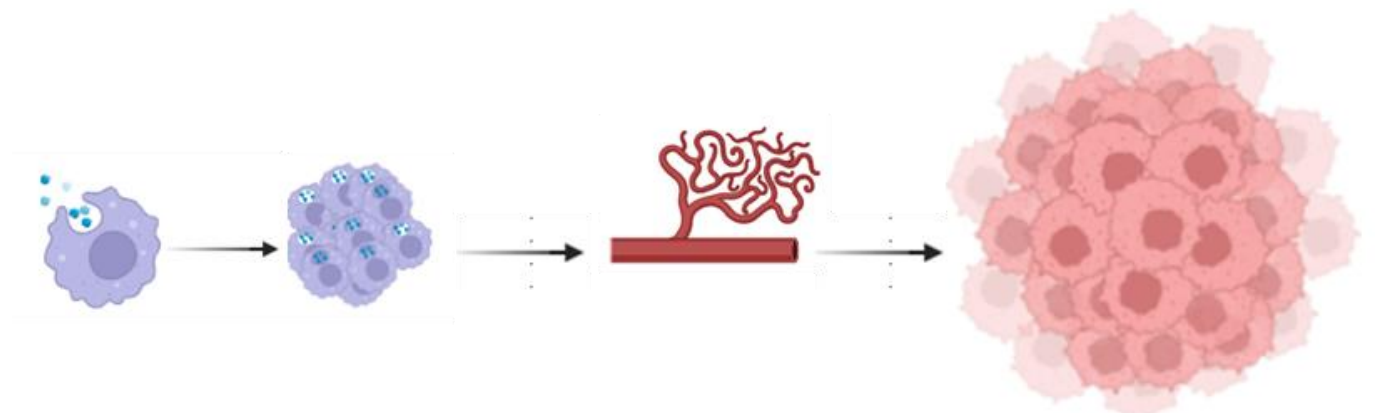


(iii) the lymphatic system also plays an important role in their distribution



(iv) the main elimination is renal and/or hepatic, being hepatic in this case due to nanoparticle size

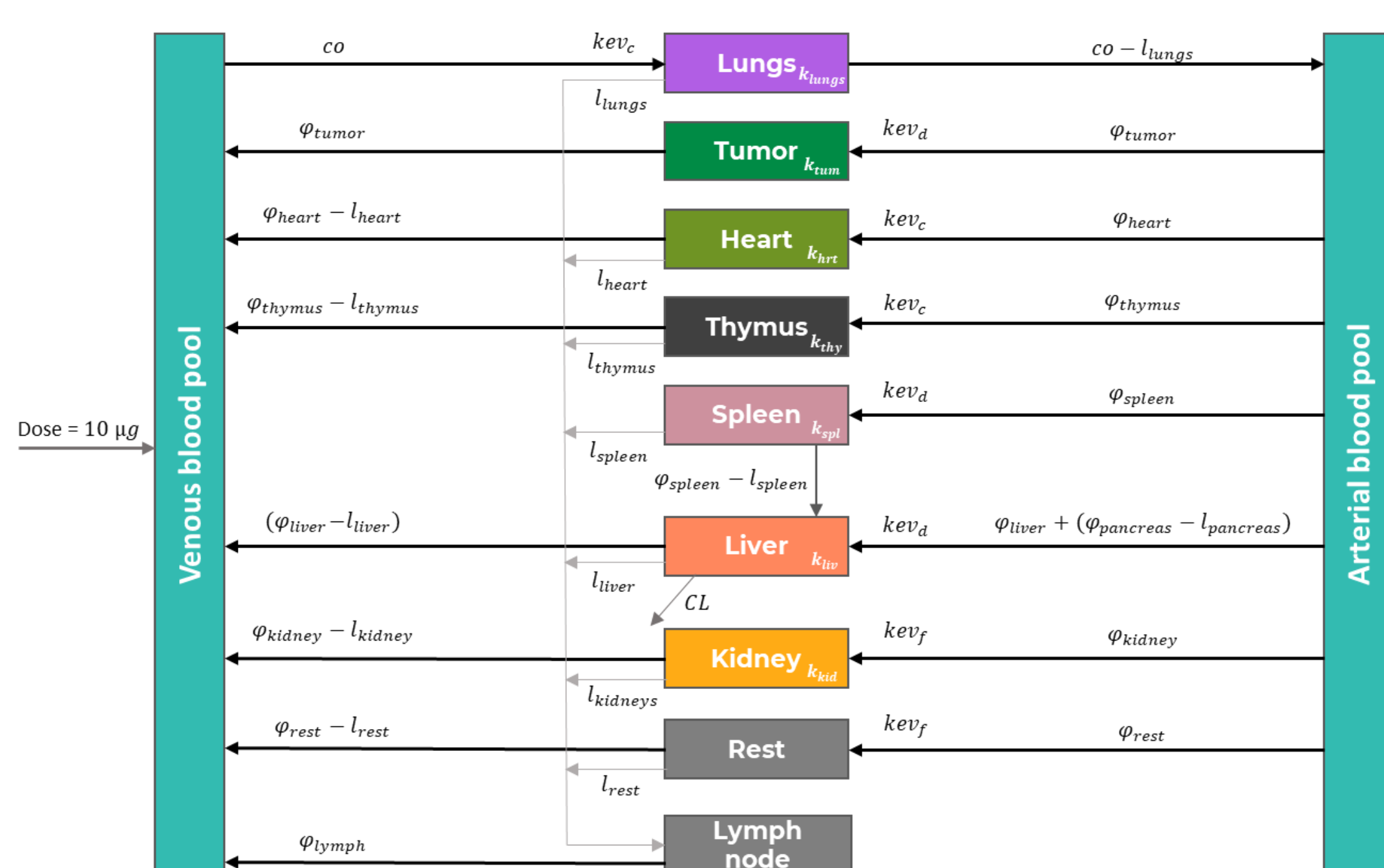
(v) tumor compartment specific characteristics need to be taken into account, such as Enhanced Permeability and Retention effect (EPR)



## MATERIAL & METHODS

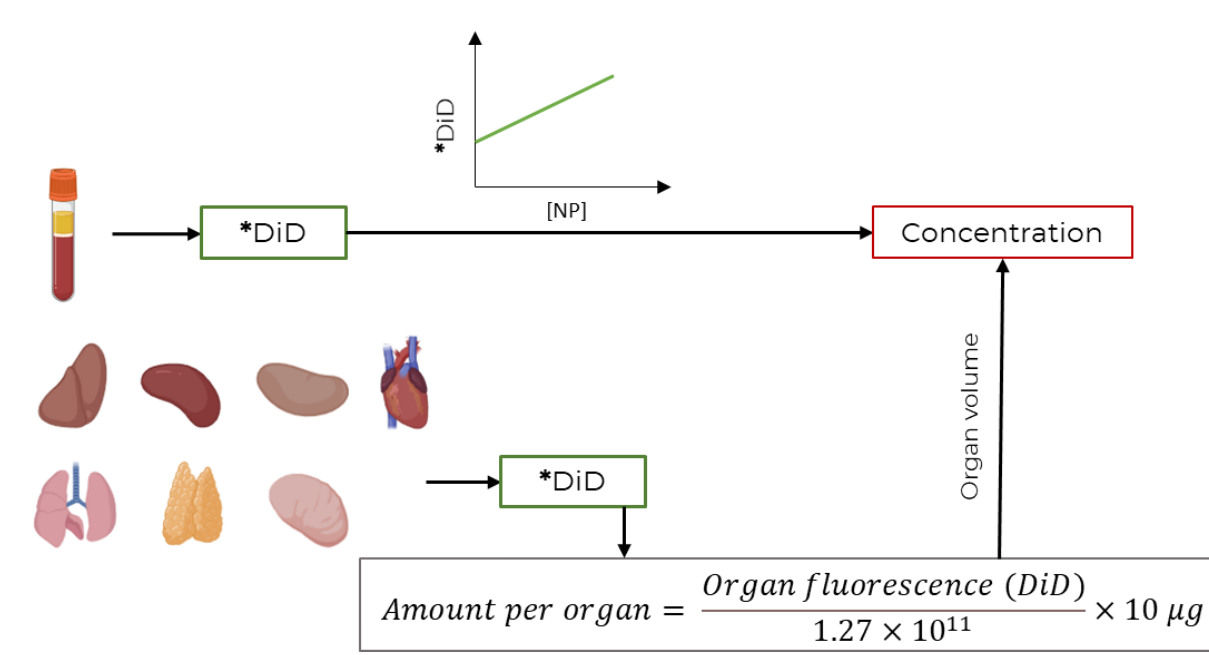
6-week-old 24 C57B6/J mice (50 % female) were inoculated with MC38 cells. Seven days after inoculation, when tumor volume was approximately 50 mm<sup>3</sup>, mice were treated with P60 10 µg of loaded NP dyed with \*DiD (fluorescent probe). Samples of **blood** and different tissues (**lung**, **tumor**, **heart**, **thymus**, **spleen**, **liver**, and **kidneys**) were taken at 0.25, 1, 3, 6, 24 and 48 hours. A Naïve Pool Approach (NPA) was employed for model development, carried out in the software Monolix2024R1 (Simulix).

### PBPK SCHEME



**Figure 1.** Schematic representation of the permeability-limited distribution physiological pharmacokinetic final model. This PBPK model contains two different blood compartments (venous blood pool and arterial blood pool) and nine compartments connected by organ blood flow ( $\phi_{org}$ ) and each of them connected to the lymph node by organ lymph flow ( $\phi_{org}$ ). It is worth mentioning that the flow to the lungs is the cardiac output ( $co$ ). Furthermore, the different partition coefficients for each organ are denoted as  $k_{org}$ . As mentioned, three constants were included, (i)  $ke_v$ , (ii)  $ke_d$  and (iii)  $ke_v$  referencing the nature of the vascular endothelium of the corresponding organ. The clearance mechanism is denoted by hepatic elimination expressed as  $CL$ .

### RAW DATA



### PHYSIOLOGICAL RELATED PARAMETERS

**Table 1.** Physiological related parameter for a 6-week-old mouse of 20 g weight. Both blood flow and lymph flow values for spleen include the sum of flows through pancreas and spleen.

Organ	Volume (mL)	Blood flow (mL/h)	Lymph flow (mL/h)
Total	20	328.2	0.652
Arteries	0.228	328.2	-
Heart	0.095	168	0.042
Kidneys	0.34	78	0.001
Liver	1.3	21	0.042
Lungs	0.1	328.2	0.034
Lymph	0.113	1.65	0.006
Spleen	0.1	8.52	0.017
Thymus	0.035	-	-
Tumor	0.05	-	-
Veins	0.524	328.2	-
Rest	16.985	201.55	0.652

### PBPK EQUATIONS

$$\frac{dA_{artery}}{dt} = (co \times \frac{C_{lungs}}{k_{lungs}}) - \sum (\phi_{organ} \times C_{arteria} \times ke_{v,org})$$

$$\frac{dA_{vein}}{dt} = \sum (\phi_{organ} - l_{organ}) \times \frac{C_{organ}}{k_{organ}} + ((\phi_{rest} - l_{rest}) \times C_{rest}) + (\phi_{lymph} \times C_{lymph}) - (co \times C_{vein} \times ke_v)$$

$$\frac{dA_{lungs}}{dt} = (co \times C_{vein} \times ke_v) - ((co - l_{lungs}) \times \frac{C_{lungs}}{k_{lungs}}) - (l_{lungs} \times C_{lungs})$$

$$\frac{dA_{organ}}{dt} = (\phi_{organ} \times C_{artery} \times ke_{v,organ}) - ((\phi_{organ} - l_{org}) \times \frac{C_{organ}}{k_{organ}}) - (l_{organ} \times C_{organ})$$

$$\frac{dA_{liver}}{dt} = ((\phi_{pancreas} - l_{pancreas}) + \phi_{liver}) \times C_{artery} \times ke_{v,d} + ((\phi_{spleen} - l_{spleen}) \times \frac{C_{spleen}}{k_{spleen}}) - ((\phi_{liver} - l_{liver}) \times \frac{C_{liver}}{k_{liver}}) - (CL \times C_{liver}) - (l_{liver} \times C_{liver})$$

$$\frac{dA_{tumor}}{dt} = (\phi_{tum} \times C_{artery} \times ke_{v,d}) * (1 - \frac{C_{tumor}}{C_{tum} + k_{tum}})$$

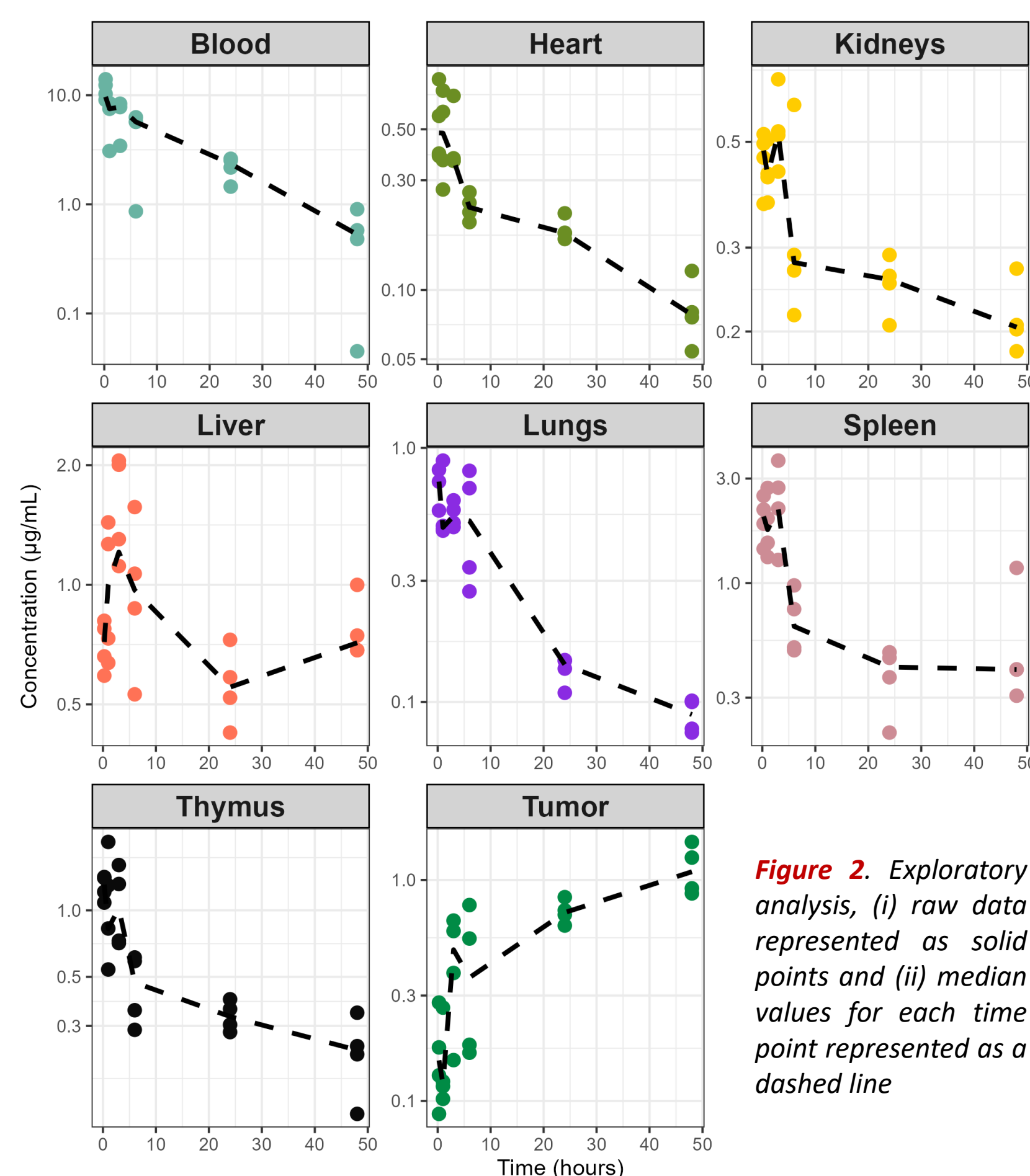
$$\frac{dA_{rest}}{dt} = (\phi_{rest} \times C_{artery} \times ke_{v,r}) - ((\phi_{rest} - l_{rest}) \times C_{rest}) - (l_{rest} \times C_{rest})$$

$$\frac{dA_{lymph}}{dt} = \sum (l_{organ} \times C_{organ}) - (\phi_{lymph} \times C_{lymph})$$

$A_i$  is defined as drug amount in tissue  $i$

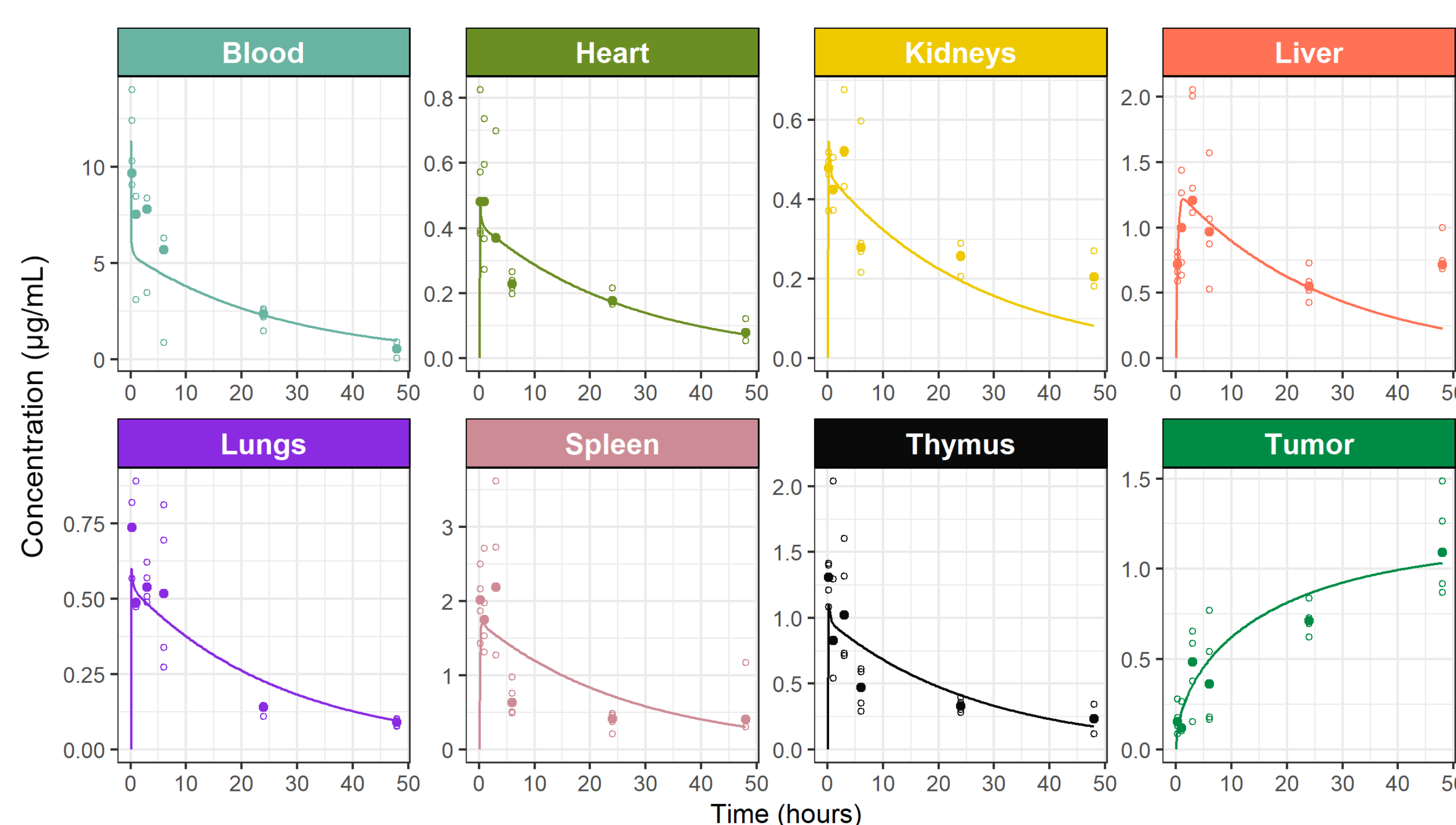
## RESULTS

### EXPLORATORY ANALYSIS



**Figure 2.** Exploratory analysis, (i) raw data represented as solid points and (ii) median values for each time point represented as a dashed line

### MODEL PERFORMANCE



**Figure 3.** Visual representation of the (i) raw data (observations) as empty points, (ii) median of raw data values for each time and organ as solid points, and (iii) the typical profile of the predicted concentrations for each organ represented as solid lines.

**Table 2.** Estimates of PBPK model parameters

Parameter	VALUE	R.S.E. (%)
$k_{tum}$	0.33	10.9
$k_{thy}$	1.29	3.34
$k_{hrt}$	0.54	7.92
$k_{kid}$	3.81	5.89
$k_{tum}$	0.026	15.8
$k_{spl}$	0.11	9.63
$k_{liv}$	0.046	2.97
$ke_v$	0.17	7.49
$ke_d$	0.028	10.1
$ke_v$	3.51	14.8
$\phi_{tum}$ (mL/h)	0.0027	6.63
$\phi_{tum}$ (mL/h)	6.66	23
$CL$ (mL/h)	1.32	0.347

## CONCLUSIONS & FUTURE PERSPECTIVES

1. A Physiologically-Based Pharmacokinetic Model for nanoparticles has been developed in tumor bearing mice describing adequately the time course of NP concentrations in blood, different organs and tumor, after single intravenous administration.
2. The model developed, in addition to the standard components of physiological organ volumes and blood flows, incorporated the following features: (i) the lymphatic system, (ii) an accumulation mechanism for tumor compartment, and (iii) size dependent NP extravasation. This is justified by physiological factors such as the endothelium of the vessels or EPR, which have been identified as potential influencers of distribution.
3. Partition coefficients ranged from 0.026 (tumor) to 3.81 (kidney). In tumor the observed accumulation of NP was described using a saturation Michaelis-Menten model accounting for a continuous increase in concentration, governed by a constant, until reaching a saturation point.

## BIBLIOGRAPHY

