Additional File 1

Predicting the effect of different folate doses on [68Ga]Ga-PSMA-11 organ and tumor uptake using physiologically based pharmacokinetic modelling

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PBPK model development for folate

Folic acid was used as the compound to represent folate intake. A simplified description of the metabolism was included because of model simplicity, where folic acid was metabolized by one dummy enzyme to one main metabolite 5-methylhydrofolate (5-MTHF). This dummy enzyme is further referred to as dihydrofolate reductase (DHFR), which is the main enzyme responsible for folic acid metabolism. Concentration-time profiles of folic acid and 5-MTHF after oral administration of folic acid (400 μg and 5 mg) were available from literature [1, 2]. Also for 5-MTHF, plasma concentration-time plots after administration of oral 5-MTHF (436 μg and 5 mg) were obtained from literature [1, 2]. Both a low and high dose input were evaluated, to capture two important processes, namely the saturation of the metabolizing enzyme and the increased unchanged folic acid excretion with higher dosages (due to exceeding renal capacity for reabsorption) [3-7]. Data was obtained by using PlotDigitizer (version 3) [8] and these data was used to eventually evaluate and optimize model predictions.

Input parameters for folic acid and 5-MTHF were based on literature values. The molecular weight of folic acid was 441.4 g/mol, while lipophilicity was -2.5 [9]. For 5-MTHF these parameters were 459.5 g/mol and -0.5, respectively. Binding to plasma proteins for folic acid was fixed to 50%, while for 5-MTHF this was 60% [10, 11]. Renal clearance of 5-MTHF was optimized using parameter optimization based on 5-MTHF observations after 5-MTHF dosing. For folic acid renal clearance was scaled to 50% fraction excreted in urine after dosing of 5 mg, assuring that with low doses only a small amount appeared in the urine [12]. The Proton-Coupled Folate Transporter (PCFT), Reduced Folate Carrier (RFC), Multidrug Resistance Proteins 3 (MRP3), Folate Receptor α (FOLR) and Organic Anion Transporter K1 (OAT-K1) were incorporated in the PBPK model to describe active absorption, efflux, transmembrane transport, renal excretion and tubular reabsorption. Transporter locations and transporter-related parameters were initially based on literature and parameters were optimized based on the obtained plasma data [13, 14]. A built-in Monte Carlo algorithm was used for parameter identification to optimize selected input parameters based

on observed patient data. Initial and optimized affinity parameters and the reference concentrations for these transporters are provided in Table S1.

PBPK model evaluation

Model predictions were evaluated for both 5-MTHF and folic acid administration in low and high dose. For 5-MTHF, plasma concentration-time predictions accurately described observed concentrations after both low (436 µg) and high (5 mg) oral dose administration (see Figure S1A). For folic acid, both parent and metabolite concentrations were predicted and evaluated for the low dose (400 µg), while for high dose (5 mg) only metabolite data was available for evaluation. Model predictions after folic acid administration showed adequate description of the observed patient data derived from literature (see Figure S1B), although predictions show a slightly higher and earlier peak in concentration for folic acid.

Table S1 Overview of most important input parameters that were fixed or fitted to describe folic acid and

5-MTHF biodistribution using the PBPK model.

	Fixed or fitted (*) value	Reference
Reference concentration		
- PCFT	1.56 µmol/L *	
- RFC	3.59 µmol/L *	
- MRP3	2.52 µmol/L*	
- FOLR	2.65 nmol/L *	
- OAT-K1	1.00 µmol/L	
- DHFR	0.04 µmol/L *	
K_m (folic acid)		
- PCFT	1.00 µmol/L	[15]
- FOLR1	1.00 nmol/L	[7, 16]
- MRP3	0.0501 mmol/L *	[17]
- OAT-K1	1.00 µmol/L	
- DHFR	0.05 µmol/L *	[18]
V_{max} (folic acid)		
- PCFT	2.49 µmol/L/min *	
- FOLR1	2.90 nmol/mL/min	[7, 16]
- MRP3	36.4 nmol/mL/min *	[17]
- OAT-K1	2.00 µmol/L/min *	
- DHFR	0.02 nmol/mL/min	[18]
K_m (5-MTHF)		
- RFC	1.00 µmol/L	[17, 19]
- FOLR1	10 nmol/L	[7, 16]
- MRP3	0.422 µmol/L *	[17, 19]
V_{max} (5-MTHF)		
- RFC	1.29 µmol/L/min *	[19]
- FOLR1	2.90 nmol/mL/min	[7, 16]
- MRP3	0.0222 nmol/mL/min *	[17]
Renal clearance 5-MTHF	1.22 mL/min/kg *	

Abbreviations: 5-MTHF: 5-methyltetrahydrofolate; FOLR: folate receptor α; K_m : Michaelis-Menten

constant; MRP3: multidrug resistance protein 3; OAT-K1: organic anion transporter K1; PCFT: proton-

coupled folate transporter; RFC: reduced folate carrier; V_{max}: maximum velocity of the enzymatic reaction.
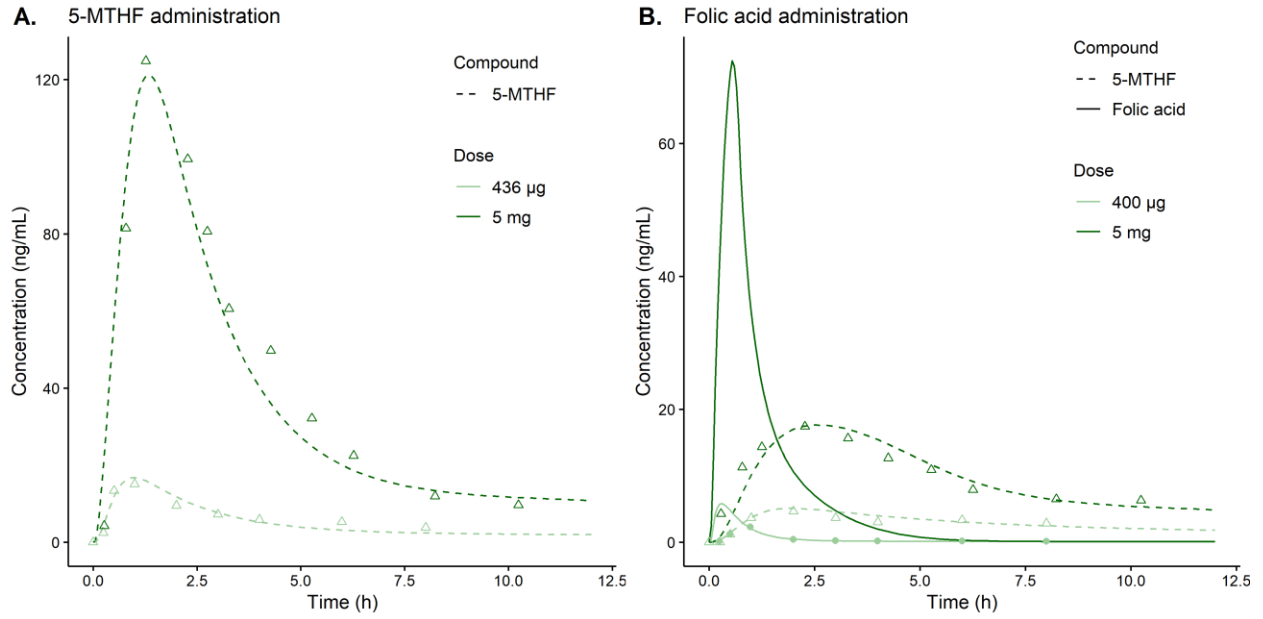
Fig. S1 Concentration-time predictions (lines) vs observations (dots and triangles) for 5-MTHF and folic acid after administration of a low (light green) and high dose (dark green) of both 5-MTHF (A) and folic acid (B).

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