SUPPLEMENTAL MATERIALS AND METHODS

Integration Plot Analysis

Cerebral, hepatic, renal and pulmonary uptake clearances ($CL_{uptake,brain}$, $CL_{uptake,liver}$, $CL_{uptake,kidney}$ and $CL_{uptake,lung}$) of ^{11}C-erlotinib were measured from 0.3 to 3.5 min after tracer injection for brain, liver and kidney and from 0.6 to 4.5 min after tracer injection for lung using the previously described integration plot method (*1,2*) and the following equation:

$$\frac{X_{t,organ}}{C_{t,blood}} = CL_{uptake} \times \frac{AUC_{0-t,blood}}{C_{t,blood}} + V_E,$$

where $X_{t,organ}$ is the amount of radioactivity per gram tissue in brain, liver, kidney or lung at time t, and $C_{t,blood}$ is the radioactivity concentration in the left ventricle of the heart at time t. $AUC_{0\text{-}t,blood}$ represents the area under the concentration-time curve in the left ventricle of the heart from time 0 to time t. CL_{uptake} can be obtained by performing linear regression analysis of a plot of $X_{t,organ}/C_{t,blood}$ *versus* $AUC_{0\text{-}t,blood}/C_{t,blood}$ and calculating the slope of the regression line. The unit of CL_{uptake} is mL blood per min per gram tissue (mL/min/g tissue) and it thus corresponds to K_1 from kinetic modelling of PET data (*2*). V_E is the y-intercept of the integration plot.

Biliary excretion clearance (CL_{bile}) of ^{11}C-erlotinib was measured from 8.8 to 65 min after tracer injection using the integration plot method and the following equation:

$$X_{t,intestine} = CL_{bile} \times AUC_{0-t,liver} + V_E$$

where $X_{t,intestine}$ is the amount of radioactivity per gram tissue in the intestine (including gall bladder) at time t. $AUC_{0\text{-}t,liver}$ represents the area under the concentration-time curve in the liver from time 0 to time t. CL_{bile} can be obtained be performing linear regression analysis of a plot of $X_{t,intestine}$ *versus* $AUC_{0\text{-}t,liver}$ and calculating the slope of the regression line. V_E is the y-intercept of the integration plot.

Analysis of Metabolites and Plasma Protein Binding

FVB/N wild-type mice, pretreated either with vehicle (*n*=6) or elacridar (10 mg/kg, formulated in 20% (v/v) aqueous ethanol solution) (*n*=4) at 2 h before radiotracer injection, and *Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$ mice pretreated with vehicle (*n*=3) were injected under isoflurane anesthesia with ^{11}C-erlotinib (28±9 MBq, 2±3 nmol, 0.1 mL, *n*=13) as an i.v. bolus over 1 min. At 25 min after ^{11}C-erlotinib injection a terminal blood sample was withdrawn under isoflurane anesthesia from the retro-orbital sinus vein and the animals were sacrificed by cervical dislocation. Additionally whole brain, liver and gall bladder were removed and urine was collected. Blood was centrifuged to obtain plasma, unlabelled erlotinib (1 mg/mL in water/acetonitrile; 5/1, v/v, 0.5 µL per µL plasma) was added and proteins were precipitated by the addition of acetonitrile (1 µL per µL plasma). To homogenized brain and liver tissue unlabelled erlotinib (1 mg/mL in water/acetonitrile; 5/1, v/v, 100 µL) was added and proteins were precipitated by the addition of acetonitrile (200 µL per brain, 2 mL per liver). Recovery of radioactivity from plasma, brain and liver was >90%. Urine and bile were precipitated by the addition of acetonitrile (1 µL per µL urine, 2 µL per µL bile). All solutions were vortexed and then centrifuged (12,000 g, 5 min, 21°C). Each supernatant (plasma, brain, liver, urine, bile, 3 µL each) and diluted radiotracer solution as reference were spotted on thin-layer chromatography (TLC) plates (silica gel 60F 254 nm, 10 x 20 cm; Merck, Darmstadt, Germany) and plates were developed in ethyl acetate/ethanol (6/4, v/v). Detection was performed by placing the TLC plates on multisensitive phosphor screens (Perkin-Elmer Life Sciences, Waltham, MA). The screens were scanned at 300 dpi resolution using a PerkinElmer Cyclone® Plus Phosphor Imager (Perkin-Elmer Life Sciences). The retention factor (R_f) of ^{11}C-erlotinib was 0.50. Metabolites in the urine were detected at R_f values of 0.43, 0.33 and 0.17, and in plasma at R_f values of 0.40 and 0.34.

Plasma protein binding of ^{11}C-erlotinib was determined in quadruplicate by ultrafiltration. In brief, ^{11}C-erlotinib (0.85 MBq) was added to 1-mL mouse plasma samples (Medical University of Vienna, Himberg, Austria) spiked with vehicle solution (2 µL acetonitrile/water 3/7, v/v) or 2 µg/mL elacridar hydrochloride or 2 µg/mL or 20 µg/mL erlotinib hydrochloride followed by incubation at 37°C for 20 min. All unlabelled compounds were added to plasma samples in 2 µL

acetonitrile/water (3/7, v/v). Amicon Ultra 0.5 mL centrifugal filters (Merck KGaA, Darmstadt, Germany) were preconditioned by adding unlabelled erlotinib hydrochloride solution in acetonitrile/water (3/7, v/v) (10 mg/mL, 200 µL) and centrifugation (12,000 g, 10 min, 25°C) in order to prevent adsorption of ^{11}C-erlotinib to the filters. After incubation, 200 µL plasma aliquots were transferred into the preconditioned Amicon filters followed by centrifugation (12,000 g, 30 min, 25°C). Filters and tubes were separately measured in a gamma counter and the percentage of ^{11}C-erlotinib that was not bound to plasma proteins was determined as percentage of radioactivity in the ultrafiltrate relative to the sum of radioactivity in the filter and ultrafiltrate.

SUPPLEMENTAL TABLE 1.

Uptake and Excretion Clearances of ^{11}C-Erlotinib *

	Wild-type	Wild-type after elacridar †	Wild-type pharmacological dose ††	*Abcb1a/b*$^{(-/-)}$	*Abcg2*$^{(-/-)}$	*Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$	*Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$ pharmacological dose ††
$CL_{uptake,brain}$ (mL/min/g tissue)	0.017±0.004	0.090±0.007	0.076±0.009	0.030±0.005	0.028±0.006	0.078±0.013	0.120±0.006
$CL_{uptake,liver}$ (mL/min/g tissue)	0.734±0.106 §	0.572±0.024	0.400±0.028	0.623±0.114	0.756±0.102	0.802±0.074	0.372±0.022
$CL_{uptake,lung}$ (mL/min/g tissue)	0.014±0.007	0.056±0.005	0.053±0.012	0.026±0.006	0.020±0.003	0.024±0.006	0.063±0.013
$CL_{uptake,kidney}$ (mL/min/g tissue)	0.340±0.021 ¶	0.240±0.046	0.193±0.016	0.275±0.012	0.345±0.052	0.401±0.043	0.168±0.012
CL_{bile} (mL/min/g tissue)	0.025±0.005	0.021±0.003	0.005±0.002	0.019±0.002	0.011±0.002	0.009±0.002	0.007±0.001

* Stated are the mean ± SD uptake ($CL_{uptake,organ}$) and biliary excretion clearances (CL_{bile}) of ^{11}C-erlotinib in different organs determined with the integration plot method. For statistical differences between groups see Fig. 2C, Fig. 5 and Supplemental Fig. 3.

† Animals were pretreated i.v. with elacridar (10 mg/kg) at 20 min before ^{11}C-erlotinib injection.

†† Animals were co-injected with a pharmacological dose of unlabelled erlotinib (10 mg/kg).

§ Hepatic blood flow rate in mice is 1.0 mL/min/g tissue (*3*). Therefore $CL_{uptake,liver}$ in wild-type mice corresponds to 73% extraction in a single transit.

¶ Renal blood flow rate in mice is 4.1 mL/min/g tissue (*3*). Therefore $CL_{uptake,kidney}$ in wild-type mice corresponds to 8% extraction in a single transit.

SUPPLEMENTAL TABLE 2.

Metabolism of ^{11}C-Erlotinib *

	Wild-type †	Wild-type after elacridar ††	*Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$ †
n	6	4	3
Plasma	80±9	75±14	54±12
Brain	93±14	100 §	92±4
Liver	57±18	36±15	26±16
Bile	41±24	29±9	48±21
Urine	1±2	0 ¶	4±6

* Stated is the mean ± SD percentage of unchanged ^{11}C-erlotinib in different tissues determined with radio-thin-layer chromatography at 25 min after injection of ^{11}C-erlotinib.

† Animals were pretreated i.v. with elacridar vehicle at 2 h before ^{11}C-erlotinib injection.

†† Animals were pretreated i.v. with elacridar (10 mg/kg) at 2 h before ^{11}C-erlotinib injection.

§ Radiolabelled metabolites below limit of detection.

¶ ^{11}C-erlotinib below limit of detection.

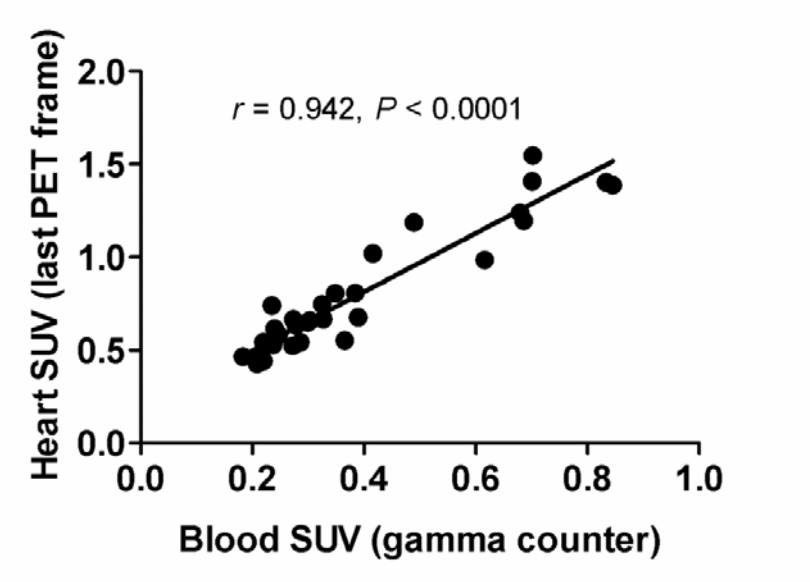
SUPPLEMENTAL TABLE 3.

Percentage of Unbound ^{11}C-Erlotinib in Plasma *

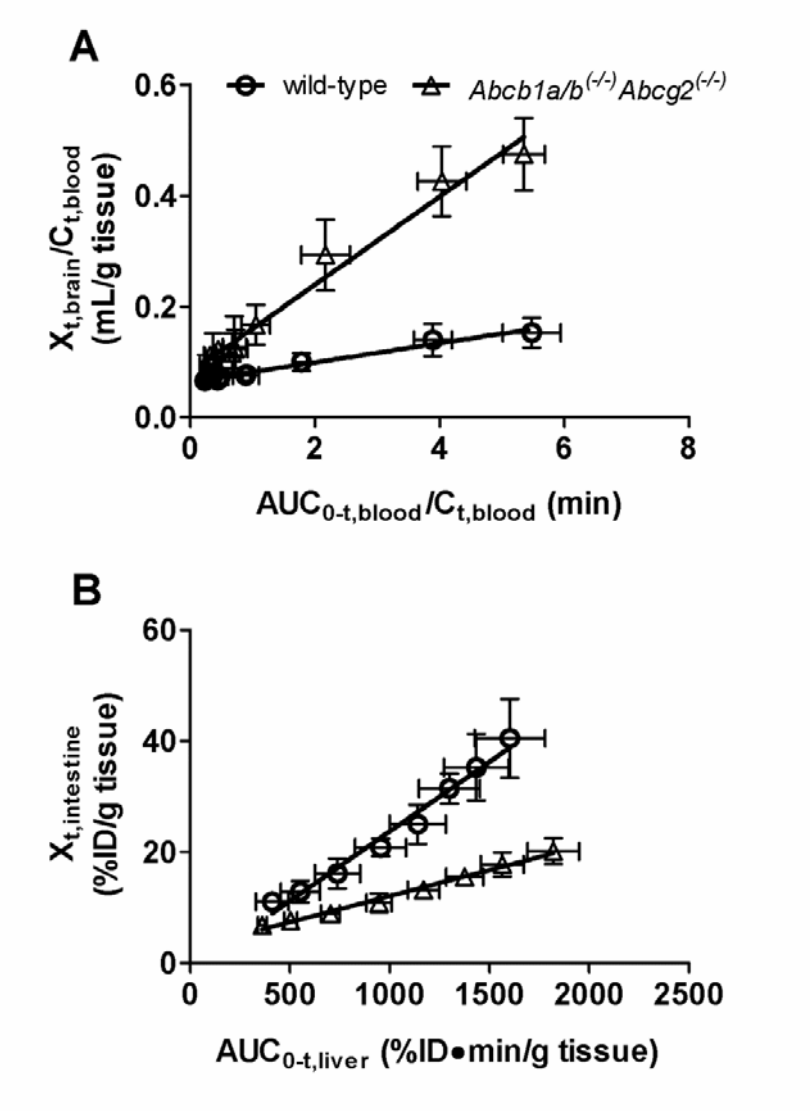
Control	Elacridar (2 µg/mL) †	Erlotinib (2 µg/mL) †	Erlotinib (20 µg/mL) †
0.22±0.03	0.32±0.03	0.18±0.04	0.27±0.03

* Stated is the mean ± SD percentage of ^{11}C-erlotinib that was not bound to plasma proteins determined by ultrafiltration in quadruplicate. No significant differences were found between groups (one-way ANOVA with Bonferroni's multiple comparison test).

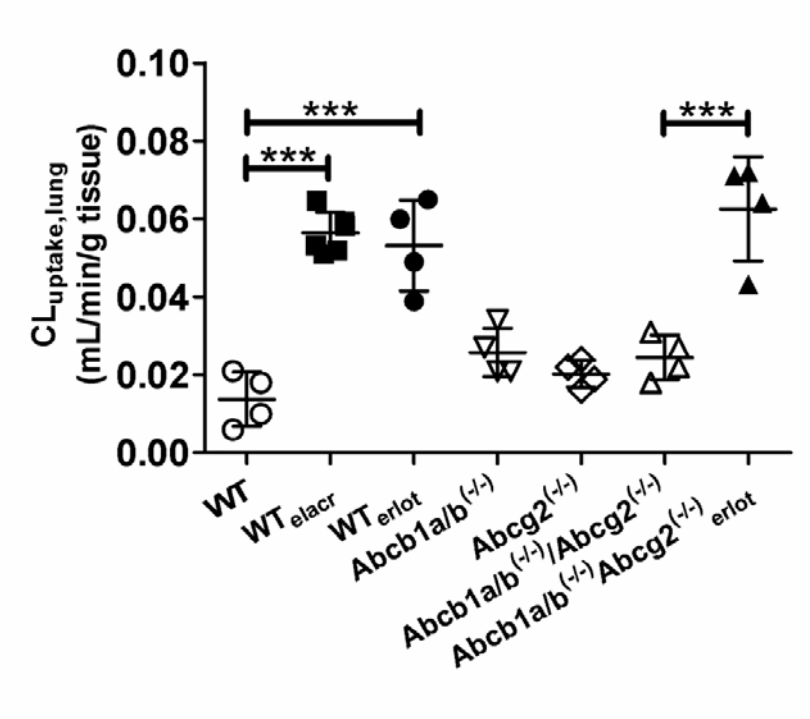
† Plasma samples were spiked with unlabelled compounds in acetonitrile/water (2 µL, 3/7, v/v).



SUPPLEMENTAL FIGURE 1. Correlation of radioactivity concentration (SUV) in the left ventricle of the heart measured in the last PET time frame (70-90 min) with radioactivity concentration (SUV) in venous blood collected at the end of the PET scan measured in a gamma counter. Solid line represents linear regression fit (*r* = Pearson correlation coefficient).



SUPPLEMENTAL FIGURE 2. Mean ± SD integration plots to estimate cerebral uptake clearance (A) and biliary excretion clearance (B) in wild-type and *Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$ mice. See "Materials and methods" for definition of variables used in integration plot analysis. The uptake and excretion clearances correspond to the slope of the linear regression lines. For estimation of cerebral uptake and biliary excretion clearances, data from 0.3-3.5 min and 8.8-65 min after radiotracer injection, respectively, were used. %ID = percent injected dose.



SUPPLEMENTAL FIGURE 3. Pulmonary uptake clearances of ^{11}C-erlotinib in wild-type mice (WT), wild-type mice pretreated with elacridar (10 mg/kg, 2 h before PET) (WT_{elacr}), wild-type mice co-injected with a pharmacological dose of unlabelled erlotinib (10 mg/kg) (WT_{erlot}), *Abcb1a/b*$^{(-/-)}$ mice, *Abcg2*$^{(-/-)}$ mice, *Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$ mice and *Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$ mice co-injected with a pharmacological dose of unlabelled erlotinib (10 mg/kg) (*Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$)$_{erlot}$). (***, P<0.001, one-way ANOVA with Bonferroni's multiple comparison test).

REFERENCES

1. Shingaki T, Hume WE, Takashima T, et al. Quantitative Evaluation of mMate1 function based on minimally invasive measurement of tissue concentration using PET with [(11)C]metformin in mouse. *Pharm Res.* 2015;32:2538-2547.
2. Takano A, Kusuhara H, Suhara T, et al. Evaluation of in vivo P-glycoprotein function at the blood-brain barrier among MDR1 gene polymorphisms by using ^{11}C-verapamil. *J Nucl Med.* 2006;47:1427-1433.
3. Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res.* 1993;10:1093-1095.