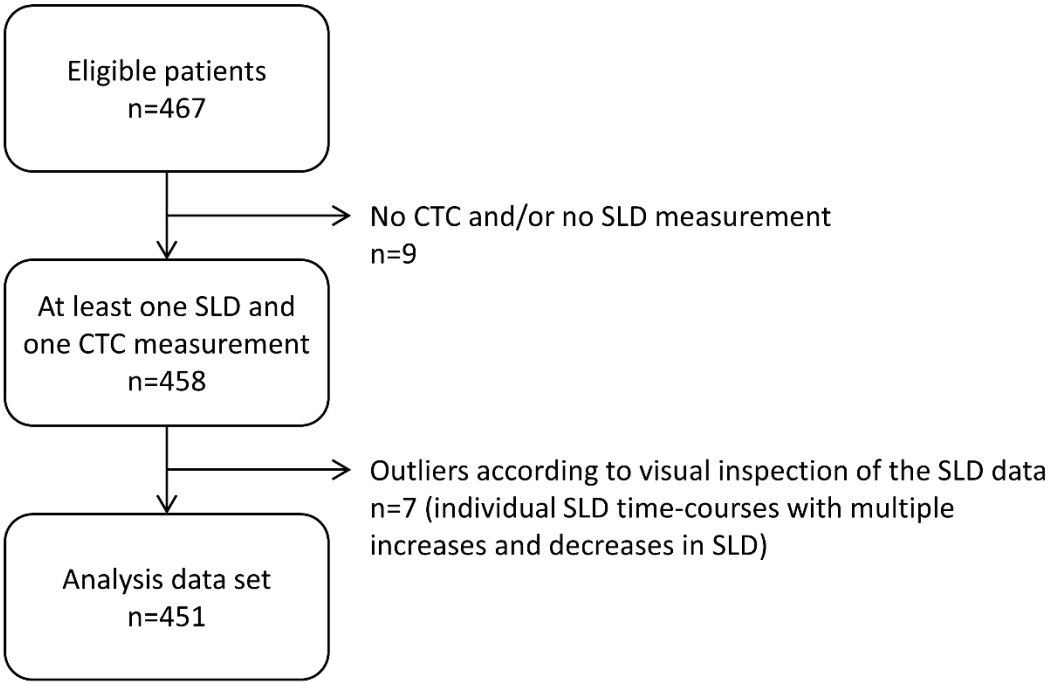
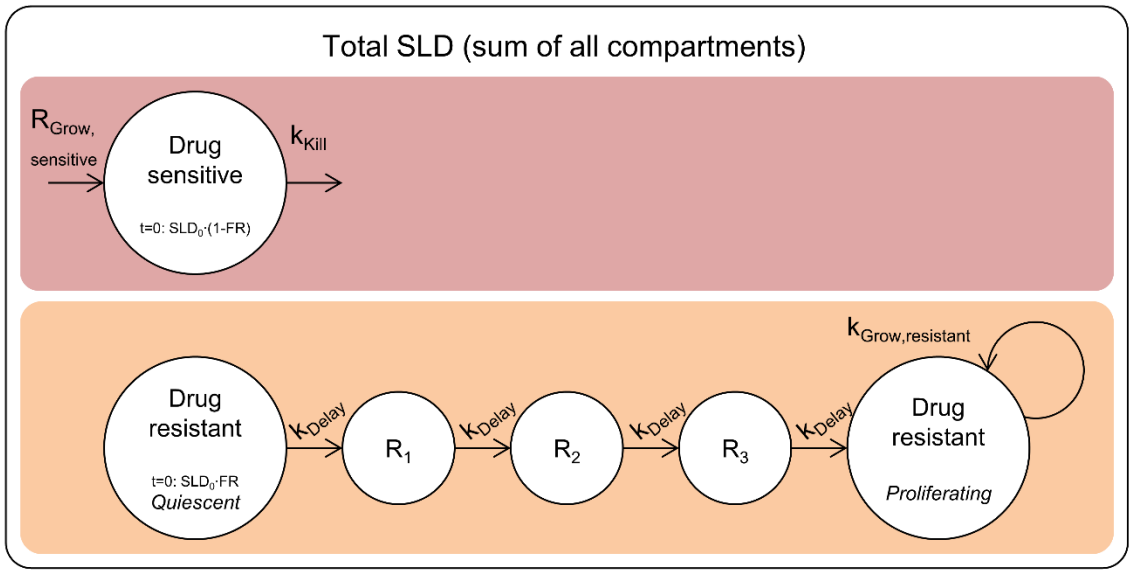


Supplementary Figure 1

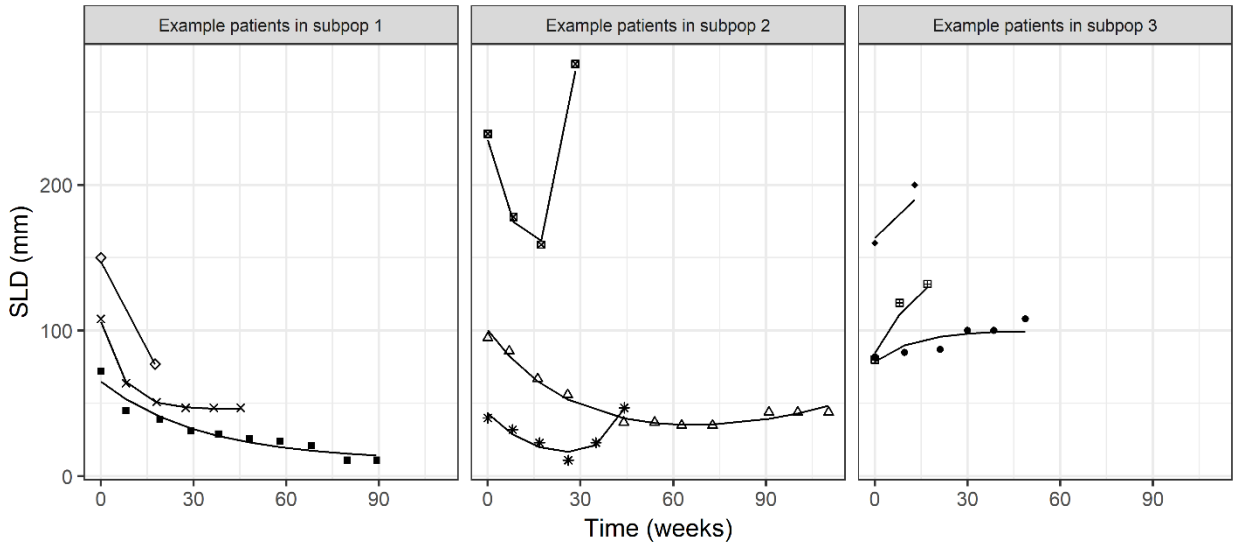
Summary of number of patients and reasons for exclusion from the analysis data set.



Supplementary Figure 2

Schematic representation of the tumor size model. The total SLD is a sum of all compartments in the pink (drug sensitive fraction) and orange (drug resistant fraction) boxes.

FR: fraction drug-resistant tumor of SLD_0, k_{Delay}: transit compartment delay rate constant from quiescent to tumor to drug-resistant tumor, $k_{Grow,resistant}$: growth rate constant of drug-resistant fraction, k_{Kill}: tumor kill rate constant, R_{1-3}: transit compartments 1-3, $R_{Grow,sensitive}$: growth rate constant of drug-sensitive fraction, SLD_0: baseline SLD



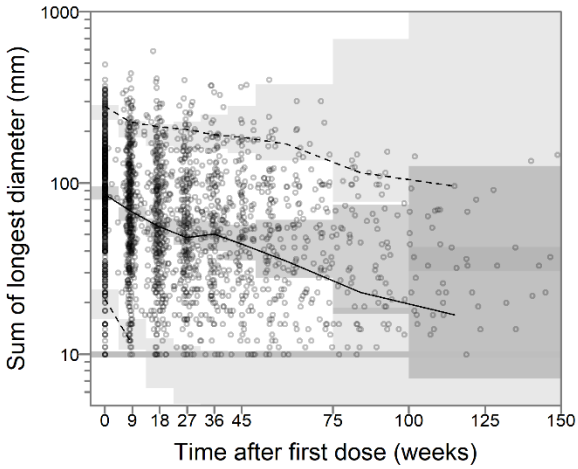
Supplementary Figure 3

Individual tumor size predictions in the final tumor size model (lines) and observed tumor sizes (dots) for 9 example patients (indicated by the shape of the dots), 3 in each subpopulation.

Patients in subpopulation 1: slow transit compartment delay rate constant from quiescent to drug-resistant tumor and slow growth rate constant of drug-sensitive fraction

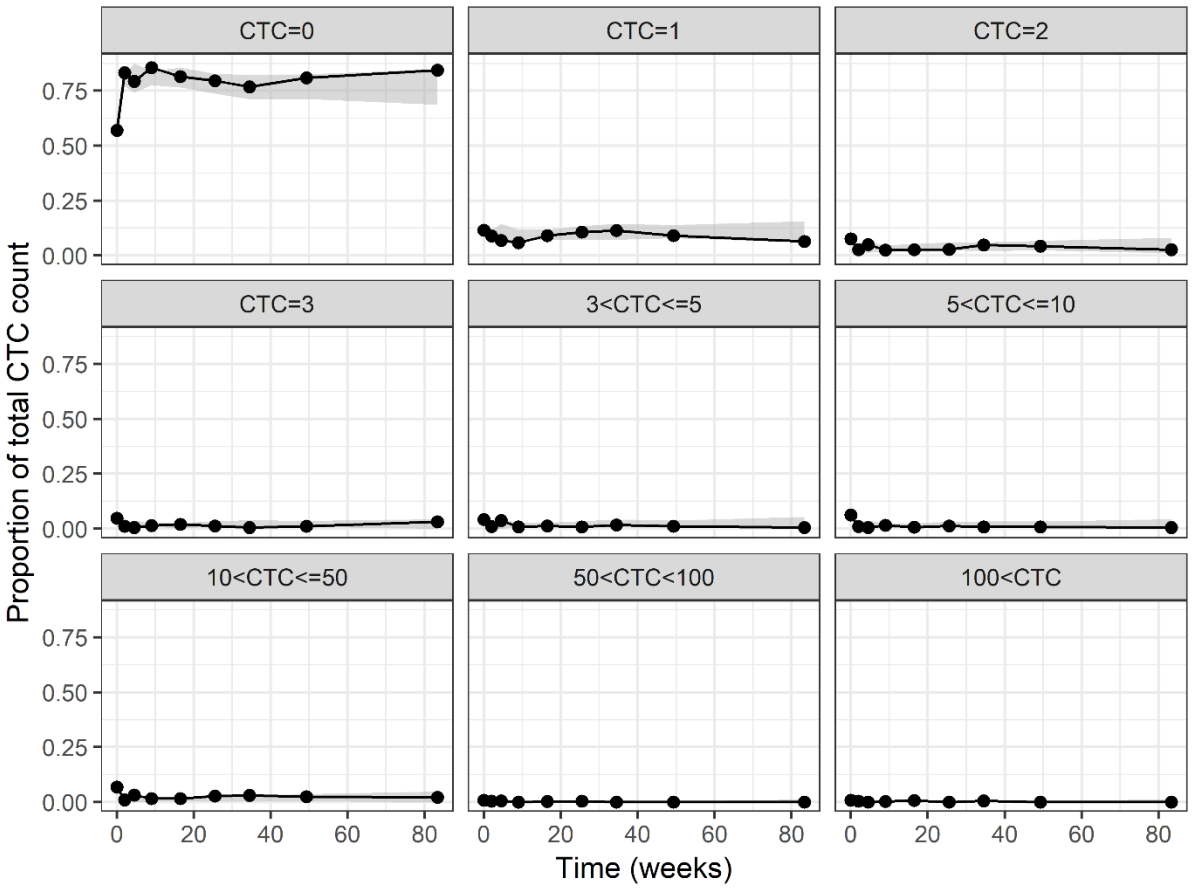
Patients in subpopulation 2: fast transit compartment delay rate constant from quiescent to drug-resistant tumor and slow growth rate constant of drug-sensitive fraction

Patients in subpopulation 3: slow transit compartment delay rate constant from quiescent to drug-resistant tumor and fast growth rate constant of drug-sensitive fraction



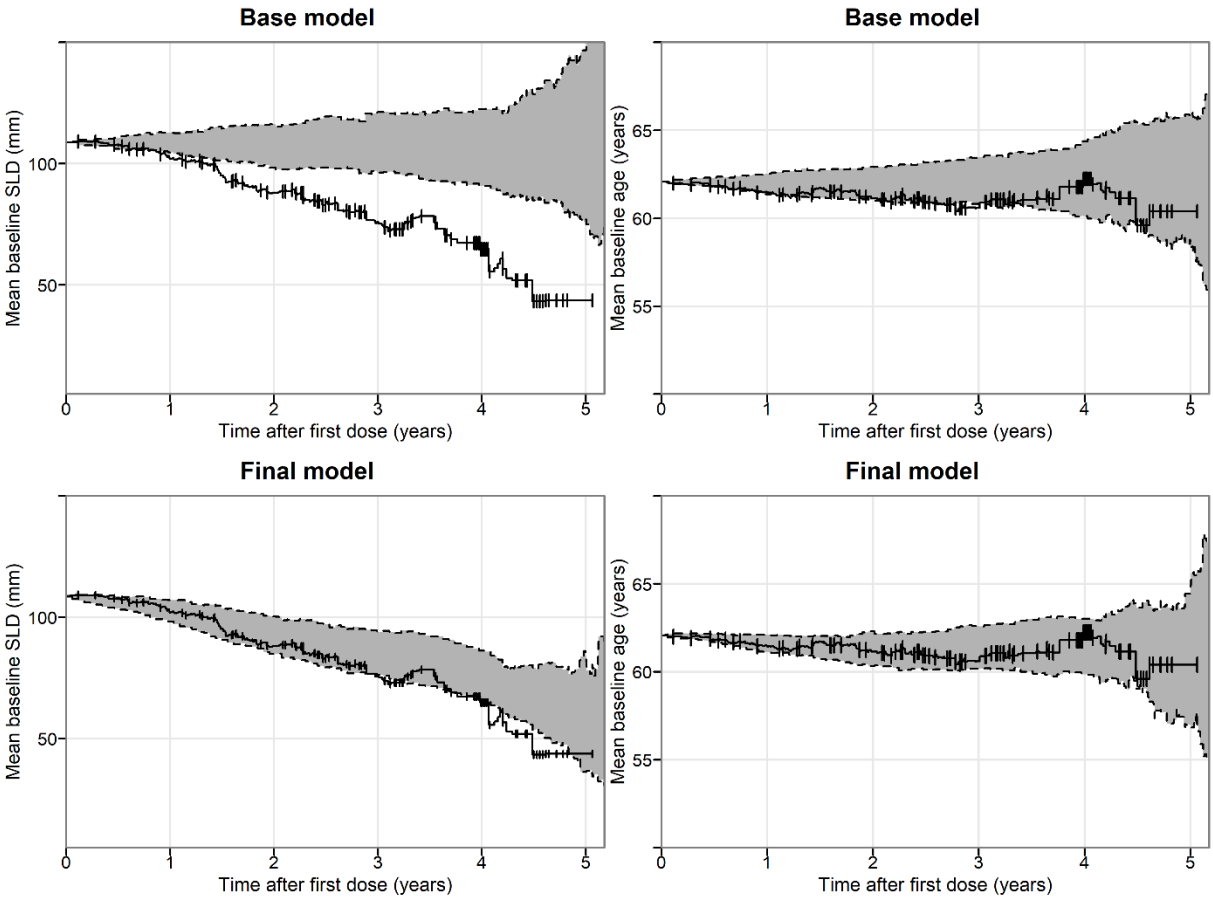
Supplementary Figure 4

VPC of the tumor size model taking dropout into account. The solid and dashed lines in the left panel represent the observed median and 5th (lower line) and 95th (upper line) percentiles of the data, respectively. The shaded areas in the left panel are the corresponding 95% CIs of the simulated data, derived from 200 simulations from the final tumor size model. The grey horizontal line represents and SLD of 10 mm.



Supplementary Figure 5

VPC of the proportions of CTC count equal to 0, 1, 2, 3, >3 and ≤5, >5 and ≤10, >10 and ≤50, >500 and ≤100 and >100 over time in the final CTC model. The dots represent the observed proportions (connected with a line) and the shaded areas are the 95% CI of the simulated data, derived from 200 simulations from the final CTC model.



Supplementary Figure 6

KMMC VPCs of the base (top plots) and final (bottom plots) OS model. The observed mean BTS and age (black lines), in comparison to the 95% CI based on 100 simulations from the base and final models (shaded areas), of patients remaining in the study over time are illustrated in the left and right plots, respectively. Vertical lines indicate censored events.

Supplementary methods

Perl-speaks-NONMEM (PsN) version 4.8.10 supported model execution and evaluation (1). Data management and model output were handled in R (https://www.R-project.org) together with the R-based packages Xpose version 4 (1) and ggplot2 version 3.0.0 (www.ggplot2.org). Pirana version 2.9.4 was used to establish run-records (1).

Sequential estimation methods similar to the individual pharmacokinetic parameters approach (IPP) and population pharmacokinetic parameters and data (PPP&D) method were applied when estimating CTC- and OS-related parameters (2,3). The individual SLD parameters were used when exploring tumor size-related metrics in the CTC and OS models while population CTC parameters were fixed during estimation of OS parameters and the corresponding CTC data was kept in the analysis data set allowing estimation of individual CTC parameters. Due to model instability it was not possible to apply PPP&D for the tumor size model parameters. Tumor sizes below 10 mm (n=219) were set to 5 mm (4).

The residual unexplained variability in the tumor size model was evaluated using proportional or combined proportional and additive error models. Interindividual variability (IIV) was allowed to be included in the tumor size and CTC model parameters and was in general exponential (or additive for parameters estimated on logit scale). Box-cox, logit, t-distributed and heavy-tail transformations were explored when indicated by graphical diagnostics in cases of reasonably low shrinkage (5).

The ΔOFVs, used to guide model building, are nominally χ^2 distributed for nested models (i.e., reduced and full models) and the degrees of freedom (DF) equal to the additional number of parameters. Significance levels of $p<0.05$ and $p<0.01$) correspond to a ΔOFV ($OFV_{reduced.model}$-$OFV_{full.model}$) of 3.84 and 6.64, respectively, for an addition of one DF. Parameter uncertainty (i.e., relative standard error, RSE) were generated using the sampling importance resampling (SIR)

approach implemented in PsN (6). Visual predictive checks (VPCs) and Kaplan-Meier VPCs (KM VPCs), based on 200 or 100 simulations, respectively, were used to evaluate the predictive performance of the models by comparing the observed data to the 95% confidence interval (CI) based on simulated data. A continuous VPC was used for the tumor size model and the CTC model was evaluated with categorical VPCs. Kaplan-Meier mean covariate VPCs (KMMC VPCs) were produced for included continuous predictors to evaluate their appropriateness (7).

References

1. Keizer R, Karlsson M, Hooker A. Modeling and Simulation Workbench for NONMEM: Tutorial on Pirana, PsN, and Xpose. CPT Pharmacomet Syst Pharmacol. 2013;2:1–9.

2. Zhang L, Beal SL, Sheiner LB. Simultaneous vs. sequential analysis for population PK/PD data I: best-case performance. J Pharmacokinet Pharmacodyn. 2003;30:387–404.

3. Wade J, Karlsson M. Combining PK and PD data during population PK/PD analysis. Abstract 139. PAGE 8 (1999).

4. Beal SL. Ways to fit a PK model with some data below the quantification limit. J Pharmacokinet Pharmacodyn. 2001;28:481–504.

5. Petersson KJF, Hanze E, Savic RM, Karlsson MO. Semiparametric distributions with estimated shape parameters. Pharm Res. 2009;26:2174–85.

6. Dosne A-G, Bergstrand M, Karlsson MO. An automated sampling importance resampling procedure for estimating parameter uncertainty. J Pharmacokinet Pharmacodyn. 2017;44:509–20.

7. Hooker A, Karlsson M. The Kaplan-Meier Mean Covariate plot (KMMC): a new diagnostic for covariates in time-to-event models. Abstract 2564. PAGE 21 (2012).

Supplementary Table 1 Baseline characteristics of the 451 patients included in the analysis data set

Continuous	**Median (range)**
Tumor size (mm)	86 (10-494)
CTC count	0 (0-351)
Age (years)	63 (28-84)
Categorical	**Number of patients (%)**
Arm	
Chemotherapy and bevacizumab	228 (51)
Chemotherapy and bevacizumab and cetuximab	223 (49)
Lactate dehydrogenase	
Normal	258 (57)
Increased	191 (42)
Missing	2 (0)
Prior chemotherapy	
Yes	57 (13)
No	394 (87)
Resection of primary tumor	
Yes	340 (75)
No	104 (23)
Missing	7 (2)

Supplementary Table 2 ΔOFV for the explored drop-out predictors in the first step

Predictor	**ΔOFV**
Progressive disease[1]	505
Normalized baseline tumor size	203
Relative change from baseline tumor time-course	9.09
Time	125

[1]20% and at least 5 mm increase in tumor size