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Pharmacogenetics of sunitinib in metastatic renal cell carcinoma

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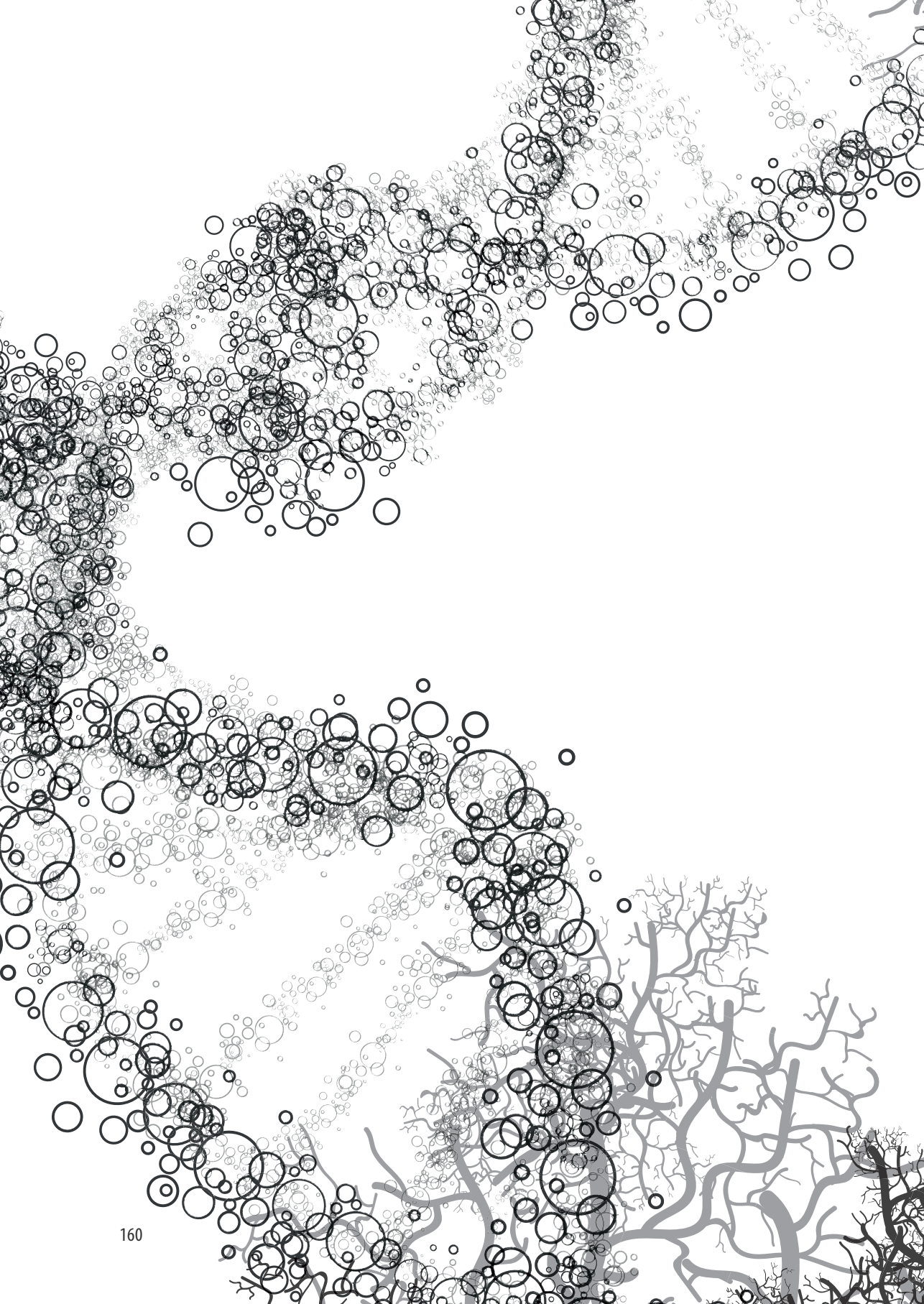


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CHAPTER 8

Population modelling integrating pharmacokinetics, pharmacodynamics, pharmacogenetics and clinical outcome in sunitinib-treated cancer patients

Diekstra MH*, Fritsch A*, Kanefendt F*, Swen JJ, Moes DJAR, Sörgel F, Kinzig M, Stelzer C, Schindele D, Gauler T, Hauser S, Houtsma D, Roessler M, Moritz B, Mross K, Bergmann L, Oosterwijk E, Kiemeny B, Guchelaar H-J, Jaehde U.

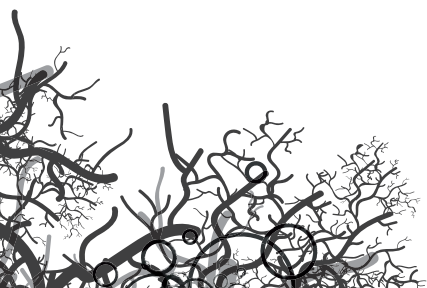
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ABSTRACT

The tyrosine kinase inhibitor sunitinib is used as first-line therapy in patients with metastasized renal cell carcinoma (mRCC), given in fixed-dose regimens despite its high variability in pharmacokinetics. Inter-individual variability of drug exposure may be responsible for differences in response. Therefore, dosing strategies based on pharmacokinetic and pharmacodynamics (PK/PD) models may be useful to optimize treatment. Plasma concentrations of sunitinib, its active metabolite SU12662 and the soluble VEGF receptors sVEGFR-2 and sVEGFR-3 were measured in 26 mRCC patients within the EuroTARGET project and 21 metastasized colorectal cancer (mCRC) patients from the C-II-005 study. Based on these observations, PK/PD models with potential influence of genetic predictors were developed and linked to time-to-event (TTE) models. Baseline sVEGFR-2 levels were associated with clinical outcome in mRCC patients, whereas active drug pharmacokinetics seemed to be more predictive in mCRC patients. The models provide the basis of PK/PD-guided strategies for the individualization of anti-angiogenic therapies.

INTRODUCTION

Sunitinib is a multi-target tyrosine kinase inhibitor (TKI), which is successfully used in the treatment of metastasized renal cell carcinoma (mRCC), gastrointestinal stromal tumors (GIST) and other solid tumor types. Sunitinib inhibits the vascular endothelial growth factor receptors (VEGFR-1, 2 and 3), the platelet-derived growth factor receptor (PDGFR) α and β , among other tyrosine kinases.^{1,2} CYP3A4 converts sunitinib into its active *N*-desethyl metabolite (SU12662) and subsequently into inactive metabolites. The elimination half-life of sunitinib is 40–60 hours and 80–110 hours for SU12662. An increased exposure to sunitinib is associated with improved survival but also with an increased risk for adverse events.^{3,4}

The individual response to sunitinib is highly variable: some patients experience severe toxicity and need dose reductions or even cessation of therapy whereas others show no response at all when using the same dose. Biomarker testing prior to start or during therapy may help provide the individual patient with the most effective treatment and the lowest possible risk of adverse effects. Whereas several potential biomarkers have been identified, they are not applied in clinical routine yet. However, sunitinib meets the requirements for therapeutic drug monitoring (TDM) enabling dose adjustment based on measured plasma drug concentrations.^{3–5}

Soluble VEGFR-3 (sVEGFR-3) was observed to be a potential predictive biomarker for overall survival (OS) on sunitinib treatment in a study on 303 patients diagnosed with GIST.⁶ Furthermore, VEGF-A and VEGFR-3 protein expression were associated with OS and progression-free survival (PFS), respectively, in 67 sunitinib-treated mRCC patients.⁷ Likewise, levels of VEGF, sVEGFR-2, and sVEGFR-3 were associated with objective response in 63 mRCC patients.⁸ With regard to genetic predictors, previous studies associated Single Nucleotide Polymorphisms (SNPs) in genes encoding metabolizing enzymes or transporters related to pharmacokinetics (PK) and pharmacodynamics (PD) of sunitinib with efficacy and toxicity.^{9–22}

In order to find predictive biomarkers for the clinical outcome of sunitinib, a better understanding of the relationships between sunitinib exposure, the pharmacological response, and the clinical outcomes is vital. This is part of the objectives of the European collaborative project EuroTARGET.²³ Several pharmacokinetic models for sunitinib have previously been published. Here, we used a nonlinear mixed-effects PK model for analyzing data of both mRCC and metastasized colorectal cancer (mCRC) patients in a pooled data set.^{23–26} This model was linked to pharmacodynamic models for sVEGFR-2 and sVEGFR-3 previously developed by our group.²⁷ The aim of our study was the development of pharmacokinetic models, linking sunitinib plasma concentrations to pharmacodynamic response and clinical outcome in a model-based time-to-event analysis including the identification of potential genetic predictors.

METHODS

Patient population

For the underlying PK/PD analysis, data were used from two pharmacokinetic studies which focused on sunitinib treatment in mRCC and mCRC patients.^{23,25} Both studies were designed as prospective, open label, single arm, multicenter, non-randomized studies and performed in accordance with the Declaration of Helsinki. Patients gave written informed consent to give venous blood for PK/PD analysis and genotyping taken in course of routine blood draw, and allowed the study sites to document clinical data.

The C-IV-001 study (EudraCT-No.: 2012-001415-23) was a phase IV PK/PD sub-study of the non-interventional EuroTARGET project.²³ Patients with mRCC were recruited in 9 medical centers in Germany and The Netherlands. Sunitinib doses ranged from 37.5 to 50 mg daily in the 4 weeks on/2 weeks off scheme. A patient was eligible for this study with a minimum age of 18 years, a diagnosed mRCC and a first-line treatment with sunitinib. Within the EuroTARGET project progression-free survival (PFS) was evaluated as primary endpoint.²³

The C-II-005 study (EudraCT-No: 2008-00151537) was performed to investigate the beneficial effect of sunitinib as add-on to biweekly FOLFIRI (folinate, fluorouracil, and irinotecan) in patients with mCRC and liver metastases.²⁵ Patients received a daily dose of 37.5 mg sunitinib on a 4 weeks on/2 weeks off treatment schedule. Primary endpoints were the reduction of tumor vessel permeability and blood flow determined by imaging techniques. Time to progression (TTP) was defined as a secondary endpoint. In case of toxicity sunitinib therapy was interrupted or continued after dose reduction to 25 mg per day until the symptoms disappeared.²⁵

Data collection and sampling

Clinical information was collected, especially demographic characteristics, concomitant medication, clinical response to the treatment, and toxicity. Serial blood samples were drawn, immediately centrifuged (1000 g, 4 °C, 15 min) and stored at -80 °C. In the C-IV-001 study, up to 12 plasma samples were collected within three cycles during routine check-ups. Except for a mandatory baseline sample before treatment start, each centre was free to develop a schedule according to their specific clinical routine. In the C-II-005 study, plasma samples were collected within two cycles at baseline, day two of each cycle and afterwards approximately every two weeks, always before sunitinib intake.

Plasma concentrations of sunitinib and SU12662 were determined using high-performance liquid chromatography coupled with mass spectrometry (LC-MS/MS, MDS SCIEX API 5000 triple quadrupole mass spectrometer; Applied Biosystems/MDS SCIEX, Thornhill, Ontario, Canada). Between-run precision and accuracy ranged from 1.6 to 6.1% and 0.2 to 9.1% for sunitinib and from 1.1 to 5.3% and -0.1 to 6.2% for SU12662,

respectively.²⁸ sVEGFR-2 concentrations were determined by commercially available ELISA kits (R&D Systems, Minneapolis, MN). The soluble VEGFR-3 was measured using a validated immunoassay.²⁶ Within-laboratory precision and accuracy of all assays were within the acceptance criteria of the EMA²⁹ with 2.2 to 4.3% and 6.2 to 14.3% for sVEGFR-2, and 0.4 to 14.7% and -3.8 to +16.2% for sVEGFR-3, respectively. Quality control (QC) samples were analyzed in all assays and runs to determine run acceptance.

SNP selection and genotyping

The selection of SNPs was based on previously reported SNP associations ($P < 0.05$) with sunitinib treatment outcome with regard to efficacy and toxicity. Herein, we have focused on SNPs that were very likely to have an effect on VEGF or VEGF receptors, or SNPs that have a high biomarker potential because of confirmatory findings in large cohorts. 13 SNPs were selected located in *CYP3A5*, *ABCB1*, *VEGF-A*, *VEGFR-2*, *VEGFR-3*, and *IL-8*.⁹⁻²² Details are provided in **Supplementary Material 1**.

Germline DNA was isolated from whole blood samples taken at baseline (before treatment initiation), using the Chemagic™ Blood kit (PerkinElmer), and genotyping was performed using the LightCycler® 480 Real-Time PCR Instrument (Roche Applied Science, Almere, The Netherlands).

Pharmacokinetic/Pharmacodynamic modelling

Data from all patients were analyzed together using the first-order conditional estimation method with interaction implemented in NONMEM (version 7.3).³⁰ The PK/PD models were built in a sequential manner. The structure of the models is shown in **Figure 1**.

Pharmacokinetic model

The pharmacokinetic model was partially based on a semi-physiological model published by Yu et al.²⁴ This model features one-compartment model for sunitinib and a biphasic distribution for SU12662. Pre-systemic formation of SU12662 is handled via a hypothetical enzyme compartment incorporated into the central compartment of sunitinib. Central compartment and enzyme compartment are connected by an inter-compartmental clearance which was fixed to the liver blood flow. The addition of a peripheral compartment for sunitinib was tested since other published models featured this structure and the underlying data indicated a similar distribution as the active metabolite.^{3,4,27} Inter-individual variability (IIV) was initially included for all parameters and removed, if their exclusion did not significantly worsen the model fit ($P < 0.05$). A proportional, additive and a combined (additive + proportional) error model were tested for parent drug and metabolite separately to describe the residual unexplained variability (RUV).

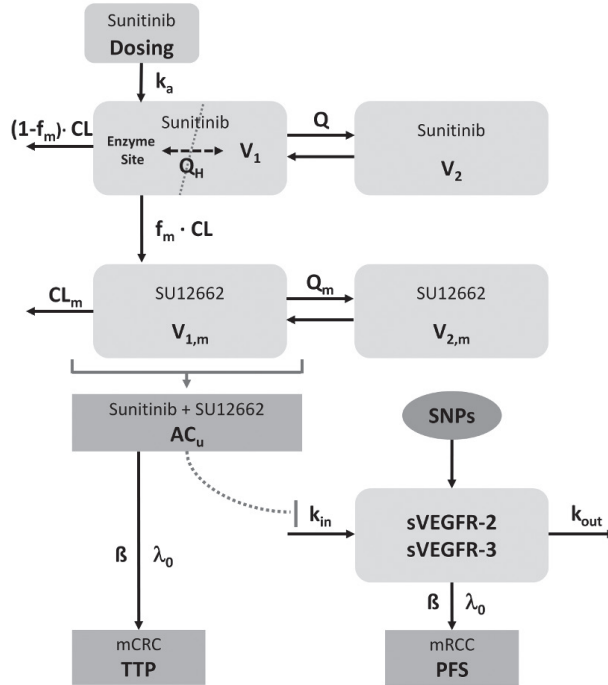


Figure 1 Model structure

Pharmacodynamic models

The concentration-time profiles of sVEGFR-2 and sVEGFR-3 were described using models developed previously by our group for healthy volunteers.²⁷ The concentration-effect relationship was described by a simple hyperbolic function (INH, **Eq. 1**) using the unbound concentration of the total active drug including SU12662 (AC_u) with a dissociation constant (k_d) fixed to 4 ng/ml obtained in vitro in a tyrosine kinase phosphorylation assay.^{27,31} Unbound concentrations were computed by assuming a protein binding of 95% for sunitinib and 90% for SU12662.³² Decreasing concentrations of the soluble receptors were described by an indirect response model with zero-order production (k_{in}) and first-order elimination (k_{out}). The inhibitory drug effect on k_{in} was included using an inverse-linear model with α as the intrinsic activity (**Eq. 2**):

$$INH = \frac{AC_u}{k_d + AC_u} \quad (1)$$

$$\frac{dsVEGFR}{dt} = k_{in} \cdot \left[\frac{1}{1 + \alpha \cdot INH} \right] - k_{out} \cdot sVEGFR \quad (2)$$

As for the PK model IIV was initially included for all model parameters and removed in case the model did not significantly worsen after exclusion.

Covariate analysis

Covariates were tested on the final models using the automated covariate search provided in PsN (Pearl speaks NONMEM, version 4.4.0).³³ In the forward inclusion step a potential covariate was significant when the OFV decreased by at least 3.84 (1 DF, $P=0.05$) and was kept in the model after the exclusion in the backwards step showed an OFV increase of at least 5.99 (1 DF, $P=0.01$). Covariates tested in the included the demographic parameters, tumor type and the preselected SNPs (see **Supplementary Material 1**).

Model qualification

Nested models were compared using the likelihood ratio test (LRT). Goodness-of-fit plots showing population (PRED) and individual predicted (IPRED) versus observed concentrations (OBS) and conditional weighted residuals (CWRES) versus IPRED or time were used to evaluate the models visually. For the final models precision of all model parameters were given as 90% confidence interval calculated by the non-parametric bootstrap approach ($N = 1,000$). Prediction-corrected visual predictive checks (pcVPCs) were generated for the patients using 1,000 simulations.³⁴ Both procedures, bootstrap and pcVPCs, were performed using PsN.³³

Sensitivity analysis

The effect of fixed parameters on the model predictions was tested by varying the respective parameters between +50 and -50% in 10% steps of the base value derived from the literature. As time of drug intake or sampling time was missing in some patients, administration time was set to 8:00 a.m., assuming that an intake in the morning is the most likely scenario. A similar approach was used for missing sampling times. Here, 12:00 p.m. was chosen, since most check-ups across study centers were scheduled around mid-day. Moreover, the influence of dosing time on parameter estimates was tested randomly varying the time of drug intake between -3 and +3 hours of the documented or imputed value.

The deviation from the original estimate was quantified by calculating the relative prediction error (RPE) and the root squared mean prediction error (RSME,%), which are defined as (Eq. 3,4)

$$RPE (\%) = \left(\frac{\theta_{new} - \theta_{base}}{\theta_{new}} \right) \quad (3)$$

$$RSME (\%) = \sqrt{(RPE)^2} \quad (4)$$

where θ_{base} denotes for the parameter estimate of the original model and θ_{new} for the new estimate under changed conditions.

Outcome modelling

Outcome analysis was performed separately for mCRC and mRCC patients using time-to-event (TTE) models based on a proportional hazard model allowing the analysis of continuous and time-dependent covariates. Different TTE distributions were tested using NONMEM.³⁴ While a constant hazard is usually a viable assumption in cancer patients due to the short survival and progression times, models with time-dependent hazards were also tested for comparison (**Eq. 5,6,7**):

Constant hazard	$h(t) = \lambda_0 \cdot e^{\beta}$	(5)
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Time-dependent hazard (Gompertz)	$h(t) = \lambda_0 \cdot e^{\beta \cdot t}$	(6)
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Time-dependent hazard (Weibull)	$h(t) = \lambda_0 \cdot e^{\beta \cdot \ln(t)}$	(7)
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Dichotomous covariates were divided by their characteristic values while continuous covariates were grouped. Statistical significance of difference between groups was determined using the log-rank test.

In addition, the Kaplan-Meier analysis as classical non-parametric method was used to determine the median progression-free survival (PFS) in mRCC and the time-to-progression (TTP) in mCRC patients.^{36,37} Kaplan-Meier analysis and Cox regression were performed using the *survival* package in R.³⁶

RESULTS

Patients

Clinical characteristics of the included patients are presented in **Table 1**. 27 mRCC patients treated with sunitinib were recruited of which one patient was excluded from the analysis due to lack of pharmacokinetic data. 28 mCRC patients were recruited of which seven patients were excluded because of missing drug administration (N=5), missing data (N=1), or uncertainty in the documentation of sunitinib intake (N=1). Thus, 26 mRCC and 21 mCRC patients treated with sunitinib were included into the combined PK/PD analysis.

Outcome analysis was performed for each tumor entity separately with regard to the different endpoints of each study using data of 24 mRCC and 21 mCRC patients. Two of the 26 mRCC patients were excluded from the outcome analysis as both received sunitinib as second-line therapy.

Moreover, 25 mRCC and 14 mCRC patients could be genotyped on the 13 selected SNPs. Here, we observed SNP call rates of 94% to 100% and 10 of 13 SNPs were in Hardy-Weinberg equilibrium (HWE) with P values >0.05 . Only the SNPs *ABCB1* rs1045642, and *VEGFA* rs699947 and rs2010963 were not in HWE with $P=0.009$, $P=0.002$, and $P=0.03$ respectively.

Table 1 Patient characteristics (median and range)

	mRCC patients (N=26)	mCRC patients (N=21)
Age (years)	64 (43-75)	61 (33-85)
Gender (m/f)	25/1	12/9
Weight (kg)	83 (65-106)	73 (57-106)
Height (cm)	180 (155-186)	172 (149-184)
BMI	25.7 (22.5-34.5)	26.0 (13.3-39.3)

Pharmacokinetic model

A PK model previously published by Yu et al. was adapted as basis for the structural model.²⁴ To allow comparison of the estimated parameters, volume and clearance (CL) parameters were, as in the reference model, allometrically scaled to a standard weight of 70 kg. Values for liver blood flow (Q_H) and the fraction metabolized (f_m) were fixed to their respective literature values as in the original model.²⁴ In contrast to the original model, a peripheral compartment for sunitinib was introduced, which improved the model significantly (difference in Objective Function Value (dOFV)=-123.98, $P<0.0001$). However, the volume of the peripheral compartment (V_2) could not be estimated with enough precision, hence the value was fixed to 588 L which was previously reported by Houk et al.^{3,4} Compared to the base model this still improved the model fit significantly (dOFV=-112.37, $P<0.0001$). The model fit significantly worsened without inter-individual variability (IIV) for sunitinib CL (dOFV=90.58, $P<0.0001$), central volume of distribution (V_1) of sunitinib (dOFV=41.97, $P<0.0001$), f_m (dOFV=134.67, $P<0.0001$) as well as V_1 of SU12662 (dOFV=18.47, $P<0.0001$). Therefore, IIV was kept in the final model for these parameters. The estimation of covariances improved the model further (dOFV=-20.34, $P<0.005$).

The sensitivity analysis did not reveal major effects on parameter estimates when the fixed parameters Q_H and V_2 (sunitinib) were varied between +50% and -50%. However,

variation of f_m resulted in a high variation (RSME of up to 50%) for clearance, inter-compartmental clearance as well as the central and peripheral volume of distribution of SU12662 which could be expected by their definition. Randomly varying dosing time between +3 and -3 hours relative to the reported or imputed value had primarily an effect on the absorption rate constant (k_a). RSME was relatively high with 36.9% for this parameter. As expected the residual error for sunitinib was also highly affected with an RSME of 25.2%.

Forward inclusion and backward elimination of potential covariates did not reveal any significant effects on the tested model parameters. In addition, no statistically significant differences of pharmacokinetic parameters between both tumor entities were found, confirming that the underlying model can be used across different tumor types. A complete list of covariates tested is provided in **Supplementary Material 1**. Final parameter estimates are shown in **Table 2**. Visual predictive checks indicated that central tendency and variability of both active compounds could be described adequately with the underlying model (**Figure 2A,B**).

Pharmacokinetic/Pharmacodynamic models

The inverse-linear model previously developed for healthy volunteers was also applicable to describe the concentration-time profile of both soluble VEGF receptors in mRCC and mCRC patients after sunitinib therapy.²⁷ The shape of the concentration-time curves of both soluble receptors was comparable and their response was highly correlated in mRCC ($r^2=0.594$, $P<0.0001$) and also mCRC patients ($r^2=0.635$, $P<0.0001$). However, the covariate analysis performed on both models revealed pharmacodynamic differences between the tumor entities. Addition of a proportional covariate effect of “tumor type” on the intrinsic activity α of sunitinib on sVEGFR-2 levels improved the model significantly ($\text{dOFV}=-7.45$, $P=0.006$). It was shown that intrinsic activity (α) was 32.8% lower in patients with mCRC compared to mRCC.

Intrinsic activity was also influenced by *VEGFR-3* rs6877011 genotype (1=CG/GG; 0=CC). Presence of the G-allele (CG and GG genotypes) showed a decreased α compared to the wildtype CC (2.31 vs 1.00 in case of mRCC patients and 1.55 vs 0.65 for mCRC patients). A decreased intrinsic activity was also observed for patients with presence of a T-allele in *ABCB1* rs2032582 (2.31 vs 1.59 in case of mRCC patients and 1.55 vs 1.07 for mCRC patients).

Final parameter estimates of both models are shown in **Table 3**. Visual predictive checks indicated that central tendency and variability of both proteins could be described adequately with the underlying models (**Figure 2C,D**).

Table 2 Population parameter estimates of the final PK model compared to the model of Yu et al.²⁴

Our study						Yu et al. ²⁴	
Parameter	Unit	Estimate (RSE, %)	IIV% (RSE, %)	Bootstrap mean	Bootstrap 90% CI	Estimate (RSE, %)	IIV% (RSE, %)
Sunitinib (parent drug)							
k_a	1/h	0.133 (34.6)	-	0.149	0.01-0.25	0.34 (10.8)	-
CL	L/h	33.9 (6.0)	30.3 (29.0)	33.92	30.76-37.53	35.7 (5.7)	33.9 (12.0)
V_1	L	1820 (6.6)	25.3 (30.3)	1812.1	1607.8-1812.2	1360 (6.0)	32.4(10.6)
V_2	L	588*	-	588*	-	-	-
Q	L/h	0.371 (18.9)	-	0.373	0.263-0.494	-	-
Q_H	L/h	80*	-	80*	-	80*	-
Residual error (proportional)	-	-0.367 (14.1)	-	-0.361	-0.450 - -0.283	0.06 (13.5)	-
SU12662 (metabolite)							
CL_m	L/h	16.5 (5.4)	-	16.5	15.0-17.9	17.1 (7.4)	42.1 (7.0)
$V_{1,m}$	L	730 (14.1)	42.9 (54.8)	713.6	545.9-872.9	635 (13.1)	57.9 (8.8)
$V_{2,m}$	L	592 (13.2)	-	604.9	481.0-737.4	388 (14.9)	-
Q_m	L/h	2.75 (24.6)	-	2.90	1.96-4.27	20.1 (32.6)	-
f_m	-	0.21*	34.6 (20.5)	0.21*	-	0.21*	-
Residual error (proportional)	-	-0.281 (10.8)	-	-0.276	-0.326 - -0.229	0.03 (14.1)	-
Correlations							
$\rho (CL, V_1)$	-	-	-	-	-	-	-
$\rho (CL, CL_m)$	-	-	-	-	-	0.53	-
$\rho (CL, V_{1,m})$	-	-0.0607 (48.3)	-	-0.0685	-0.127 - -0.019	-	-
$\rho (V_1, V_{1,m})$	-	0.0481 (51.8)	-	0.0534	0.0091-0.0996	0.48	-
$\rho (CL_m, V_{1,m})$	-	-	-	-	-	0.45	-
$\rho (CL, f_m)$	-	-0.0425 (40.8)	-	-0.0392	-0.0671 - -0.0130	-	-
$\rho (V_1, f_m)$	-	-	-	-	-	-	-
$\rho (f_m, V_{1,m})$	-	-	-	-	-	-	-

*Parameter fixed to literature value CI, confidence interval, CL, clearance of sunitinib, CL_m , clearance of the metabolite SU12262, f_m , fraction metabolized to SU12662, IIV, inter-individual variability, k_a , absorption rate constant, prop, proportional error model, Q, inter-compartmental clearance of sunitinib, Q_H , liver blood flow, Q_m , inter-compartmental clearance of the metabolite SU12662, ρ , correlation coefficient, RSE, relative standard error, V_1 , volume of the central compartment of sunitinib, $V_{1,m}$, volume of the central compartment of the metabolite SU12662, V_2 , volume of the peripheral compartment of sunitinib, $V_{2,m}$ volume of the peripheral compartment of the metabolite SU12662

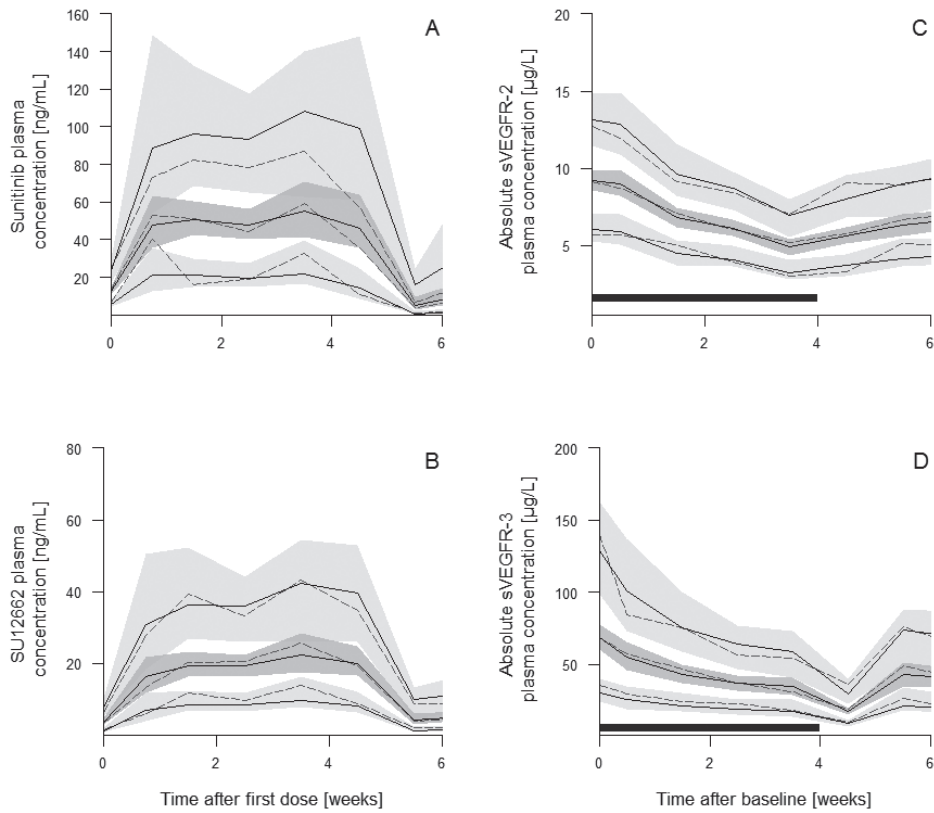


Figure 2 Prediction-corrected visual predictive checks of **A)** the final PK model of sunitinib, **B)** the final PK model of SU12662, **C)** the sVEGFR-2 model, and **D)** the sVEGFR-3 model for one treatment cycle. Solid lines indicate the estimated mean as well as the 90% prediction interval. Dashed lines show the respective observed mean and interval. Shaded grey areas represent the 90% confidence band of the predictions. The dark grey rectangle indicates the time on treatment.

Table 3 Population parameter estimates of the final PD models (sVEGFR-2, sVEGFR-3)

Parameter	Unit	Estimate (RSE, %)	IIV (RSE, %)	Bootstrap mean	Bootstrap CI (90%)
sVEGFR-2					
Baseline	µg/L	9.0 (2.9)	19.9 (21.4)	9.0	8.6-9.5
α	-	2.31 (8.8)	-	2.31	1.98-2.64
k_{out}	1/h	0.0043 (7.6)	-	0.0043	0.0038-0.0049
k_d	µg/mL	4*	-	4*	-
Residual Error	-	0.124 (6.8)	-	0.122	0.108-0.136
Tumor type on α (proportional)	-	-0.328 (24.6)	-	-0.322	-0.440- -0.186
VEGFR-3 rs6877011 on α (proportional)	-	-0.565 (25.4)	-	-0.557	-0.787- -0.319
ABCB1 rs2032582 on α (proportional)	-	-0.311 (37.9)	-	-0.307	-0.497- -0.117
sVEGFR-3					
Baseline value	µg/L	63.5 (5.9)	42.6 (24.4)	63.7	57.3-69.8
α	-	1.74 (9.8)	54.3 (43.5)	1.76	1.49-2.05
k_{out}	1/h	0.0053 (7.2)	-	0.0054	0.0047-0.0060
k_d	µg/mL	4*	-	4*	-
Residual Error	-	0.15 (6.9)	-	0.15	0.13-0.17
Tumor type on baseline value (proportional)	-	-0.642 (6.5)	-	-0.640	-0.703- -0.569
Correlations					
ρ (baseline value, α)		0.124	-	0.123	0.045-0.209

*Parameter fixed to literature value

α , intrinsic activity, CI, confidence interval, IIV, inter-individual variability, k_d , dissociation constant, k_{out} , elimination rate constant, ρ , correlation coefficient, RSE, relative standard error

Outcome model for mRCC patients

Median PFS for mRCC patients was calculated with 6.9 months (N=24). The PFS could be described by a parametric time-to-event model assuming exponentially distributed data with a baseline hazard function λ_0 of 0.0252 weeks⁻¹ (90% CI: 0.0168-0.0336). The inclusion of the measured and estimated sVEGFR-2 baseline value led to a decrease of the OFV by 4.14 or 4.67 ($P<0.05$), respectively. However, the dichotomized covariate, dividing patients into two groups with baseline values above and below the population median of 8.8 µg/L, had a stronger effect with a decrease of the OFV by -6.40 ($P<0.025$). β was estimated with 1.45 corresponding to a hazard ratio (HR) of 4.26 (with β defined as the natural logarithm of the HR). Inclusion of the active, unbound sunitinib/SU12662 concentrations resulted in an estimated β of -0.14 mL/ng indicating that a higher plasma level reduces the hazard and hence the probability of progression during treatment. However, the effect was not statistically significant (dOFV=-1.1, $P=0.29$). Likewise, plasma concentrations of sVEGFR-2 and sVEGFR-3 over time were not statistically significant

predictors of PFS either (dOFV, -3.7 and -0.99, respectively). Besides absolute plasma levels of both proteins, also the relative decrease with respect to individual baseline values predicted by the PK/PD models was tested as potential covariate. However, no significant improvement of the model fit could be observed either (dOFV=-0.31 and -0.98).

Best prediction of PFS in mRCC patients was achieved by a hazard function $h(t)$ including the dichotomized baseline value of sVEGFR-2 which was independent of the developed PK/PD models (**Eq. 8**):

$$h(t) = \lambda_0 \cdot e^{\beta \cdot \text{sVEGFR-2 baseline (dichotom.)}} \quad (8)$$

The observed Kaplan-Meier curve describing the progression-free survival function of the mRCC patients was within the predicted 90% prediction interval (PI) of 1,000 simulations and could sufficiently be described by the time-to-event model except for later time points as a result of censored data (**Figure 3A**). Final parameter estimates are shown in **Table 4**.

These findings were confirmed in a multivariate Cox regression analysis. The only covariates exhibiting a significant influence were the dichotomized baseline values of both soluble proteins (data not shown).

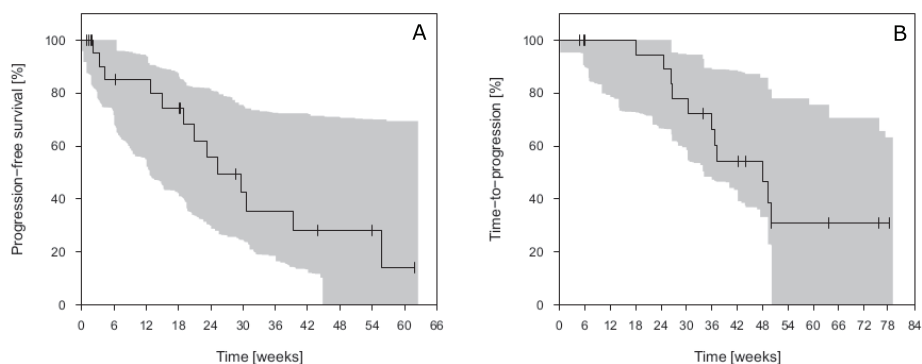


Figure 3 Prediction-corrected visual predictive checks of **A)** the final survival model for mRCC patients, and **B)** the final survival model for mRCC patients. Shaded grey areas represent the 90% prediction interval.

Table 4 Population parameter estimates of the final time-to-event models

Parameter	Covariate	Unit	Estimate (RSE,%)	Bootstrap mean	Bootstrap median	Bootstrap 90% CI
mRCC patients						
λ_0		weeks ⁻¹	0.0118 (46.3)	0.0121	0.0117	0.0038-0.0220
β	sVEGFR-2	-	1.45 (43.3)	1.57	1.49	0.71-2.68
HR	baseline value*	-	4.26	4.81	4.44	2.03-14.59
mCRC patients						
λ_0		weeks ⁻¹	0.0234	0.0256	0.0241	0.0120-0.0447
β	AC _u	ml/ng	-0.758	-0.919	-0.836	-0.366- -1.736
HR		-	0.47	0.40	0.43	0.18-0.69

*dichotomized above (1) and below (0) population median ACU, unbound active concentration (sunitinib + SU12662), β , regression coefficient, CI, confidence interval, HR, Hazard Ratio, λ_0 , baseline hazard, ρ , correlation coefficient, RSE, relative standard error

Outcome model for mCRC patients

Median time to progression (TTP) for mCRC patients was 8.4 months (N=21). Analogous to the mRCC patients, the TTP could be described by a parametric time-to-event model assuming exponentially distributed data. The baseline hazard function λ_0 was estimated with 0.0234 weeks⁻¹ (90% CI:0.012-0.042 weeks⁻¹). The inclusion of the current concentration of the unbound, active drug (AC_u) reduced the OFV by 6.07 ($P<0.05$). β was estimated to be -0.758 mL/ng corresponding to a HR of 0.47. None of the other variables describing the individual PK or biomarker response were identified to be predictive for TTP. Therefore, TTP in mCRC patients was best predicted by the pharmacokinetics of sunitinib and SU12662 with an appropriate hazard function $h(t)$ dependent on the current AC_u(t) (**Eq. 9**):

$$h(t)=\lambda_0 \cdot e^{\beta \cdot AC_u(t)} \quad (9)$$

The observed Kaplan-Meier curve describing the PFS function of the mCRC patients was within the predicted 90% prediction interval of 1,000 simulations and could sufficiently be described by the time-to-event model. However, TTP was difficult to predict for the time from one year onwards due to censored data (**Figure 3B**). Final parameter estimates are shown in **Table 4**. A multivariate Cox regression analysis confirmed these results exhibiting the AUC at steady-state (AUC_{ss}) of the unbound, active drug in combination with age as positive predictive covariates (data not shown).

DISCUSSION

In this study we successfully integrated distinct models for sunitinib in a modeling framework including PK, PD, pharmacogenetic and outcome data. The developed models adequately describe plasma concentration-time profiles of sunitinib, its active metabolite SU12662, sVEGFR-2 and sVEGFR-3 as well as clinical outcome in both tumor types. Similar models (but without pharmacogenetics) were published in patients with GIST⁶ and recently hepatocellular carcinoma³⁸ but there is no model with integrated outcome data yet published for the tumor entities investigated here.

Covariate analysis on the PK parameters did not reveal any significant findings. The significant increase of sunitinib clearance in patients with *ABCB1* rs2032582 TT (18%) found in previous studies could not be confirmed.¹⁷ Presumably this is due to the small and, with two tumor entities, relatively heterogeneous cohort. Biomarker response of sVEGFR-2 and -3 was highly associated in each tumor entity which suggested a comparable predictive value of both soluble receptors. As previously reported decreasing plasma concentrations were observed for both receptors after sunitinib administration with a subsequent increase after stop of treatment.^{8,27,39} Independent of tumor entity and dosing scheme, baseline levels are not fully recovered after a two weeks off-phase.

A difference in sVEGFR-2 response to sunitinib between mRCC and mCRC patients could be identified. However, decrease of sVEGFR-2 plasma levels relative to the individual baseline did not have a significant impact on PFS or TTP in both studies, hence the clinical relevance of this effect might be negligible. Observed baseline values of sVEGFR-3 were in the same magnitude previously reported by Motzer et al. ranging between 22.3 and 129.2 µg/L for mRCC patients. However, they were significantly higher compared to mCRC patients.⁴⁰ This finding might indicate a higher expression of this protein in mRCC patients. However, data regarding the baseline values of sVEGFR-3 in mCRC patients is sparse, since the first- and second-line treatment usually does not involve tyrosine kinase inhibitors targeting sVEGFR.⁴¹

In this study, we found that the presence of the variant G-allele in SNP rs6877011 in *VEGFR-3* was associated with a 56.5% decrease in intrinsic activity on sVEGFR-2 compared to the wild-type CC. The same *VEGFR-3* SNP was associated with a decreased PFS in an earlier study.¹² Maitland et al. associated variant G-allele carriers of *VEGFR-2* (*KDR*) rs34231037 with sVEGFR-2 baseline levels and a decline in sVEGFR-2 in response to treatment with pazopanib.⁴² We have recently found that rs34231037 variant G-allele carriers have a tendency towards a better response to sunitinib.⁴³ VEGFR-1, 2, and 3 have similar binding domains.⁴⁴ A SNP in any of the genes encoding these VEGF receptors could result in a conformation change and prevent or stimulate binding of the drug ligand to VEGF receptors, and change the ability of sunitinib to decrease sVEGFR-2 and sVEGFR-3. It is remarkable that the SNP effect of G-allele carriers of

rs6877011 in *VEGFR-3* was not found on the intrinsic activity of sunitinib on sVEGFR-3 but on VEGFR-2. Possibly, a lower activity of sunitinib on sVEGFR-3 could also affect sVEGFR-2. The conformation change may have more impact on VEGFR-2 binding affinity than VEGFR-3.

In both patient groups we succeeded in linking clinical outcome data to either pharmacodynamics (mRCC) or pharmacokinetics (mCRC). In mRCC patients, baseline levels of sVEGFR-3 and sVEGFR-2 as well as the decrease in sVEGFR-2 plasma levels over the treatment duration were previously reported to be related to clinical outcome.^{7,45} These findings could be further confirmed by this study. Whereas the effect of sVEGFR-2 decrease over time was not significant in the time-to-event analysis, patients with a substantially higher baseline value of sVEGFR-2 showed a significantly worse PFS with an estimated Hazard Ratio (HR) of 4.26. The baseline value of sVEGFR-3 had a lower influence on PFS: for patients with a sVEGFR-3 baseline above the population median of the HR was 2.38 without statistical significance ($P=0.2$). An effect of similar magnitude (HR: 2.4, 95% CI: 1.13-5.11) was reported by Harmon et al. for the same covariate.⁴⁶ In contrast, in mCRC patients, the time-to-event model showed an effect of the pharmacokinetics on TTP with precise parameter estimates. Higher exposure to sunitinib and SU12662 included as active drug concentration over time was associated with a longer TTP. Similarly, a meta-analysis with 443 cancer patients including advanced GIST, mRCC and other solid tumors suggested that an increased AUCss is associated with a longer TTP and a longer OS.⁴

It is not surprising that plasma concentrations of proteins related to the VEGF pathway seem to be more predictive for clinical outcome in mRCC patients as most renal cell carcinoma cells overexpress VEGF due to mutations in the von-Hippel Lindau gene.⁴⁷ Furthermore, sunitinib showed no additional effects in patients with colorectal cancer²⁵, which is consistent with our findings that sVEGFR-2 and -3 levels were not correlated to outcome. The lower intrinsic activity of sunitinib on sVEGFR-2 baseline levels and the overall lower plasma concentrations of sVEGFR-3 may also underline the lower dependency of colorectal carcinomas on angiogenesis, especially via VEGF signaling.

In conclusion, a semi-mechanistic PK model for sunitinib could be successfully linked to pharmacodynamic models for sVEGFR-2 and sVEGFR-3 including various genotypes. Whereas we could show that sunitinib pharmacokinetics does not differ between the two tumor entities, we found differences in pharmacodynamic response with respect to the decrease of sVEGFR-2 and sVEGFR-3 plasma concentrations during therapy. Furthermore, sVEGFR-2 baseline levels seemed to be more predictive for clinical outcome in mRCC patients in contrast to mCRC patients where active drug pharmacokinetics showed the highest impact. Nevertheless, our study provides the basis of PK/PD-guided individualization strategies for the optimization of anti-angiogenic therapies and underlines that it is quite unlikely to identify a general, tumor entity-independent biomarker for sunitinib therapy response.

STUDY HIGHLIGHTS

What is the current knowledge on the topic? There is a high inter-individual variability in response to sunitinib. Hence, predictive biomarkers are needed in order to maximize efficacy and minimize toxicity.

What question did this study address? The objective of this study was the development of pharmacokinetic models, linking sunitinib plasma concentrations to pharmacodynamic response and clinical outcome including the identification of potential genetic predictors for metastasized renal cell cancer (mRCC) and metastasized colorectal cancer (mCRC) patients.

What this study adds to our knowledge? The developed PK/PD models adequately describe plasma concentration-time profiles of sunitinib, SU12662, sVEGFR-2, sVEGFR-3, and clinical outcome showing the strength of an integrated modelling approach. Clinical response in mRCC patients is best predicted by baseline sVEGFR-2 levels, whereas in mCRC patients active drug pharmacokinetics is more predictive.

How might this change drug discovery, development, and/or therapeutics? The PK/PD models presented in this study provide a better understanding of the relationship between sunitinib exposure, pharmacological response, and clinical outcome, and are hence an important step towards finding predictive biomarkers for clinical outcome of sunitinib.

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SUPPLEMENTARY MATERIAL

Supplementary Material 1: List of covariates tested with the respective models

Pharmacokinetic model	
Gender	-
Age	-
Tumor entity (mRCC or mCRC)	-
ABCB1 rs1128503	Additive and general model (if applicable)
ABCB1 rs2032582	Additive and general model (if applicable)
ABCB1 rs1045642	Additive and general model (if applicable)
VEGFA rs699947	Additive and general model (if applicable)
VEGFA rs833061	Additive and general model (if applicable)
VEGFA rs2010963	Additive and general model (if applicable)
IL8 rs1126647	Additive and general model (if applicable)
HAP_VEGFA	Additive and general model (if applicable)
Pharmacodynamic models	
Gender	-
Age	-
Weight	-
BSA	-
Tumor entity (mRCC or mCRC)	-
ABCB1 rs1128503	Additive and general model (if applicable)
ABCB1 rs2032582	Additive and general model (if applicable)
ABCB1 rs1045642	Additive and general model (if applicable)
VEGFA rs699947	Additive and general model (if applicable)
VEGFA rs833061	Additive and general model (if applicable)
VEGFA rs2010963	Additive and general model (if applicable)
IL8 rs1126647	Additive and general model (if applicable)
HAP_VEGFA	Additive and general model (if applicable)
FLT-4 (=VEGFR3) rs6877011	Additive and general model (if applicable)
FLT-4 (=VEGFR3) rs307826	Additive and general model (if applicable)
FLT-4 (=VEGFR3) rs307821	Additive and general model (if applicable)
Time-to-event models	
Active concentration of sunitinib and SU12662	-
AUC of active substance	-
Total concentration of sunitinib and SU12662	-
AUC of total substance	-
Absolute concentration of sVEGFR-2	-
Relative concentration of sVEGFR-2	-
AUC of sVEGFR-2	-
Absolute concentration of sVEGFR-3	-
Relative concentration of sVEGFR-3	-
AUC of sVEGFR-3	-
Systolic blood pressure	-

Supplementary Material 1: Continued

Time-to-event models	
Diastolic blood pressure	-
Rel. change in syst. Blood pressure	-
Rel. change in syst. Blood pressure	-

Supplementary Material 2: NONMEM Codes**Pharmacokinetic model**

\$SUBROUTINE ADVAN6 TOL=4

\$MODEL
 NCOMP =5
 COMP = (DEPOT, DEFDOSE)
 COMP = (OBSLIV)
 COMP = (CENTRALM)
 COMP = (PERIM)
 COMP = (PERISUN)

\$PK

WT = WEIGHT
 IF (WEIGHT.EQ.-99.AND.SEX.EQ.1) WT = 83 ; ET Population mean - male
 IF (WEIGHT.EQ.-99.AND.SEX.EQ.0) WT = 75 ; ET Population mean - female
 $ASCL = (WT/70)^{0.75}$
 $ASV = WT/70$

KA = THETA(1)
 V2 = THETA(2)*ASV * EXP(ETA(1))
 QH = THETA(3)*ASCL
 CLP = THETA(4)*ASCL* EXP(ETA(3))
 CLM = THETA(5)*ASCL
 V3 = THETA(6)*ASV * EXP(ETA(2))
 Q34 = THETA(7)*ASCL
 V4 = THETA(8)*ASV
 FM = THETA(9) * EXP(ETA(4))

```

Q25    = THETA(10)*ASCL
V5      = THETA(11)*ASV

```

```

K34 = Q34/V3
K43 = Q34/V4
K25 = Q25/V2
K52 = Q25/V5

```

```

S2 = V2
S3 = V3

```

```

$DES
CLIV = (KA*A(1) + QH/V2*A(2))/(QH+CLP)

```

```

DADT(1) = -KA*A(1)
DADT(2) = QH*CLIV-QH/V2*A(2) - K25*A(2) + K52*A(5)
DADT(3) = FM*CLP*CLIV-CLM/V3*A(3)-K34*A(3) + K43*A(4)
DADT(4) = K34*A(3)-K43*A(4)
DADT(5) = K25*A(2) - K52*A(5)

```

```

$ERROR
TY = LOG(F)
IF(F.LT.0.001) TY = 0.001
IPRED = TY

```

```

IF (CMT.EQ.2) THEN ; DV log-transformed
W = SQRT(THETA(12)**2)
Y = IPRED+W*EPS(1)
ENDIF

```

```

IF (CMT.EQ.3) THEN ; DV log-transformed
W = SQRT(THETA(13)**2)
Y = IPRED+W*EPS(2)
ENDIF

```

```

IRES = DV-IPRED
DEL = 0
IF(W.EQ.0) DEL = 0.0001
IWRES = IRES/(W+DEL)

```

\$THETA

(0, 0.133)	;1 = KA
(0, 1820)	; 2 = V2
(80) FIX	; 3 = QH
(0, 33.9)	;4 = CLP
(0, 16.5)	;5 = CLM
(0, 730)	;6 = V3
(0, 2.75)	;7 = Q34
(0, 592)	;8 = V4
(0.21) FIX	;9 = FM
(0, 0.371)	;10 = Q25
(588) FIX	;11 = V5
(0,0.367)	;12 = Prop. Error Suni
(0,0.281)	;13 = Prop. Error Metab

\$OMEGA BLOCK(4)

0.0621	;1 IIV V2
0.0473 0.169	;2 IIV V3
0 -0.0613 0.088	;3 IIV CLP
0 0 -0.0421 0.113	;4 IIV FM

\$SIGMA

1 FIX ;
1 FIX ;

\$ESTIMATION SIG=2 SIGL=4 PRINT=1 METHOD=1 INTER MAXEVAL=9999 NOABORT

\$COVARIANCE PRINT=E

sVEGFR-2 PK/PD model

\$SUBROUTINE ADVAN6 TOL=4

\$MODEL

NCOMP=6
COMP =(DEPOT,DEFDOSE)
COMP =(OBSLIV)
COMP =(CENTRALM)

COMP =(PERIM)
 COMP =(PERISUN)
 COMP =(VEGFR2)

\$PK

; Covariate relationships

;;; ALFAABCR2-DEFINITION START
 IF(ABCR2.EQ.1) ALFAABCR2 = 1 ; Most common
 IF(ABCR2.EQ.-99) ALFAABCR2 = 1 ; Missing data
 IF(ABCR2.EQ.0) ALFAABCR2 = (1 + THETA(8))
 ;;; ALFAABCR2-DEFINITION END

;;; ALFASTUDY-DEFINITION START
 IF(STUDY.EQ.1) ALFASTUDY = 1 ; Most common
 IF(STUDY.EQ.2) ALFASTUDY = (1 + THETA(7))
 ;;; ALFASTUDY-DEFINITION END

;;; ALFAFLT1-DEFINITION START
 IF(FLT1.EQ.0) ALFAFLT1 = 1 ; Most common
 IF(FLT1.EQ.-99) ALFAFLT1 = 1 ; Missing data
 IF(FLT1.EQ.1) ALFAFLT1 = (1 + THETA(6))
 ;;; ALFAFLT1-DEFINITION END

;;; ALFA-RELATION START
 ALFACOV=ALFAFLT1*ALFASTUDY*ALFAABCR2
 ;;; ALFA-RELATION END

;PHARMACODYNAMICS

TVBLV2 = THETA(1) ; sVEGFR-2 Baseline
 BLV2 = TVBLV2*EXP(ETA(1))

TVALFA = THETA(2) ; Intrinsic activity

ALFA = TVALFA*ALFVACOV*EXP(ETA(2))

$$\text{TVKOUT} = \text{THETA}(3) \quad ; \text{Kout}$$
$$\text{KOUT} = \text{TVKOUT} * \text{EXP}(\text{ETA}(3))$$

$$\text{A}_0(6) = \text{BLV2}$$

$$\text{KIN} = \text{BLV2} * \text{KOUT}$$

$$\text{TVKD} = \text{THETA}(4) \quad ; \text{Dissociation constant}$$
$$\text{KD} = \text{TVKD} * \text{EXP}(\text{ETA}(4))$$

$$; \text{PHARMACOKINETICS}$$

$$\begin{aligned} \text{KA} &= 0.133 \\ \text{V2} &= \text{V2X} \\ \text{QH} &= \text{QHx} \\ \text{CLP} &= \text{CLPx} \\ \text{CLM} &= \text{CLMx} \\ \text{V3} &= \text{V3X} \\ \text{Q34} &= \text{Q34X} \\ \text{V4} &= \text{V4X} \\ \text{FM} &= \text{FMx} \\ \text{Q25} &= \text{Q25X} \\ \text{V5} &= \text{V5X} \end{aligned}$$

$$\begin{aligned} \text{K34} &= \text{Q34} / \text{V3} \\ \text{K43} &= \text{Q34} / \text{V4} \\ \text{K25} &= \text{Q25} / \text{V2} \\ \text{K52} &= \text{Q25} / \text{V5} \end{aligned}$$

$$\begin{aligned} \text{S2} &= \text{V2} \\ \text{S3} &= \text{V3} \end{aligned}$$

$$\text{\$DES}$$

$$\text{CLIV} = (\text{KA} * \text{A}(1) + \text{QH} / \text{V2} * \text{A}(2)) / (\text{QH} + \text{CLP})$$

$$\begin{aligned} \text{DADT}(1) &= -\text{KA} * \text{A}(1) \\ \text{DADT}(2) &= \text{QH} * \text{CLIV} - \text{QH} / \text{V2} * \text{A}(2) - \text{K25} * \text{A}(2) + \text{K52} * \text{A}(5) \end{aligned}$$

DADT(3) = FM*CLP*CLIV-CLM/V3*A(3)-K34*A(3) + K43*A(4)

DADT(4) = K34*A(3)-K43*A(4)

DADT(5) = K25*A(2) - K52*A(5)

;TOTAL DRUG PLASMA CONCENTRATION

CONC = A(3)/V3+A(2)/V2

;FREE DRUG CONCENTRATION; 95% PB sunitinib, 90% PB SU12662

FC= A(3)/V3*(1-0.90)+A(2)/V2*(1-0.95)

;VEGFR2

;KINASE BINDING

IF(FC.LE.0) THEN

BND=0

ELSE

BND=FC/(KD+FC)

ENDIF

;INHIBITORY SIGNAL

INH = BND

;SIGNAL FUNCTION

SF=1/(1+ALFA*INH)

;RESPONSE COMPARTMENT ABSOLUTE SVEGFR-2 LEVELS

DADT(6)= KIN*SF-KOUT*A(6)

\$ERROR

IPRE = A(6)

RBL = IPRE/BLV2

IRES = DV-IPRE

W = THETA(5)*IPRE

Y = IPRE+EPS(1)*W

DVRL = DV/BLV2

DEL = 0
IF(W.EQ.0) DEL = 1
IWRE = IRES/(W+DEL)

\$THETA
(0, 9030) ; 1 TVBLV2
(0, 2.31) ; 2 TValfa
(0, 0.00428) ; 3 THKOUT
(4) FIX ; 4 THKD
(0, 0.124) ; 5 CV
(-1, -0.565,5) ; ALFAFLT11
(-1, -0.328,5) ; ALFASTUDY1
(-1, -0.311,5) ; ALFAABCR21

\$OMEGA 0.0388 ; 1 ETABLVR2
\$OMEGA 0 FIX ; 2 ETAalfa
\$OMEGA 0 FIX ; 3 ETAKOUT
\$OMEGA 0 FIX ; 4 ETAKD

\$SIGMA 1 FIX

\$ESTIMATION SIG=2 PRINT=1 METHOD=1 INTER MAXEVAL=9999 NOABORT

\$COVARIANCE PRINT=E

sVEGFR-3 PK/PD model

\$SUBROUTINE ADVAN6 TOL=4

\$MODEL

COMP=6
COMP =(DEPOT,DEFDOSE)
COMP =(OBSLIV)
COMP =(CENTRALM)
COMP =(PERIM)
COMP =(PERISUN)
COMP =(VEGFR3)

\$PK

; Covariate relationships

```

;;; BLV3STUDY-DEFINITION START
IF(STUDY.EQ.1) BLV3STUDY = 1 ; Most common
IF(STUDY.EQ.2) BLV3STUDY = ( 1 + THETA(6))
;;; BLV3STUDY-DEFINITION END

```

```

;;; BLV3-RELATION START
BLV3COV=BLV3STUDY
;;; BLV3-RELATION END

```

```

;PHARMACODYNAMICS
;System specific

```

```

TVBLV3 = THETA(1)
BLV3 = TVBLV3*BLV3COV*EXP(ETA(1))

```

```

TVALFA = THETA(2)
ALFA = TVALFA*EXP(ETA(2))

```

```

TVKOUT = THETA(3)
KOUT = TVKOUT

```

```

A_0(6)=BLV3

```

```

KIN = BLV3*KOUT

```

```

TVKD = THETA(4);
KD = TVKD

```

```

;PHARMACOKINETICS

```

```

K12      = 0.133
V2       = V2X
QH       = QHX
CLP      = CLPX

```

CLM = CLMX
V3 = V3X
Q34 = Q34X
V4 = V4X
FM = FMX
Q25 = Q25X
V5 = V5X

K34 = Q34/V3
K43 = Q34/V4
K25 = Q25/V2
K52 = Q25/V5

S2 = V2
S3 = V3

\$DES

CLIV = (K12*A(1) + QH/V2*A(2))/(QH+CLP)

DADT(1) = -K12*A(1)
DADT(2) = QH*CLIV-QH/V2*A(2) - K25*A(2) + K52*A(5)
DADT(3) = FM*CLP*CLIV-CLM/V3*A(3)-K34*A(3) + K43*A(4)
DADT(4) = K34*A(3)-K43*A(4)
DADT(5) = K25*A(2) - K52*A(5)

;TOTAL DRUG PLASMA CONCENTRATION
CONC = A(3)/V3+A(2)/V2

;FREE DRUG CONCENTRATION; 95% PB sunitinib, 90% PB SU12662
FC= A(3)/V3*(1-0.90)+A(2)/V2*(1-0.95)

;VEGFR-3
;KINASE BINDING
IF(FC.LE.0) THEN
 BND=0
ELSE
 BND=FC/(KD+FC) ;
ENDIF

```
;INHIBITORY SIGNAL
```

```
INH = BND
```

```
;Signal Function
```

```
SF=1/(1+ALFA*INH)
```

```
;RESPONSE COMPARTMENT ABSOLUTE SVEGFR-3 LEVELS
```

```
DADT(6)= KIN*SF-KOUT*A(6)
```

```
$ERROR
```

```
IPRE   = A(6)
```

```
RBL    = IPRE/BLV3
```

```
IRES   = DV-IPRE
```

```
W      = THETA(5)*IPRE
```

```
Y      = IPRE+EPS(1)*W
```

```
DVRL   = DV/BLV3
```

```
DEL     = 0
```

```
IF(W.EQ.0) DEL = 1
```

```
IWRE    = IRES/(W+DEL)
```

```
$THETA
```

```
(0, 63500)      ; 1 TVBLV3
```

```
(0, 1.74) ; 2 TValfa
```

```
(0, 0.0054)     ; 3 TVKOUT
```

```
(4) FIX         ; 4 TVKD
```

```
(0, 0.15) ; 5 CV
```

```
( -0.642) ;6 BLV3TUMOR
```

```
$OMEGA BLOCK(2)
```

```
0.167           ; 1 ETABLVR3
```

```
0.124 0.258     ; 2 ETAalfa
```

```
$SIGMA 1 FIX
```

```
$ESTIMATION SIG=2 PRINT=1 METHOD=1 INTER MAXEVAL=9999 NOABORT
```

\$COVARIANCE PRINT=E

Time-to-Event model (mRCC)

\$SUBROUTINE ADVAN6 TOL=4

\$MODEL

NCOMP = 1

COMP = (CUMHAZ)

\$PK

TVBLHAZ = THETA(1)

BLHAZ = TVBLHAZ*EXP(ETA(1))

TVBETA = THETA(2)

BETA = TVBETA*EXP(ETA(2))

PMN = 8814.3 ; measured pop. median

IF(VG2B.GT.PMN)THEN

VGB = 1

ELSE

VGB = 0

ENDIF

\$DES

; Hazard Model

DADT(1) = BLHAZ*EXP(BETA*VGB)

\$ERROR

CHZ = A(1)

SUR = EXP(-CHZ

HAZNOW = BLHAZ*EXP(BETA*VGB)

IF(DV.EQ.1) THEN

```

      Y = HAZNOW*SUR           ; prob density function of event
ELSE
      Y = SUR                 ; censored data
ENDIF

```

```

$THETA
(0, 0.00007)      ; 1 TV_BLHAZ   - BASELINE HAZARD
(1.45)            ; 2 TV_BETA    - FACTOR

```

```

$OMEGA 0 FIX      ; 1 ETA_HAZ
$OMEGA 0 FIX      ; 2 ETA_BETA

```

```

$ESTIMATION SIG=2 SIGL=6 MAXEVAL=9999 METHOD=COND LAPLACE LIKE PRINT=1
$COVARIANCE PRINT=E

```

Time-to-Event model (mCRC)

```

$SUBROUTINE ADVAN6 TOL=4

```

```

$MODEL
NCOMP=6
COMP = (DEPOT, DEFDOSE)
COMP = (OBSLIV)
COMP = (CENTRALM)
COMP = (PERIM)
COMP = (PERISUN)
COMP = (CUMHAZ)

```

```

$PK

```

```

TVBLHAZ          = THETA(1)
BLHAZ = TVBLHAZ*EXP(ETA(1))

```

```

TVBETA           = THETA(2)
BETA             = TVBETA*EXP(ETA(2))

```

```

;PHARMACOKINETICS

```

K12 = 0.133
V2 = V2X
QH = QHX
CLP = CLPX
CLM = CLMX
V3 = V3X
Q34 = Q34X
V4 = V4X
FM = FMX
Q25 = Q25X
V5 = V5X

K34 = Q34/V3
K43 = Q34/V4
K25 = Q25/V2
K52 = Q25/V5

S2 = V2
S3 = V3

\$DES

CLIV = (K12*A(1) + QH/V2*A(2))/(QH+CLP)

DADT(1) = -K12*A(1)
DADT(2) = QH*CLIV-QH/V2*A(2) - K25*A(2) + K52*A(5)
DADT(3) = FM*CLP*CLIV-CLM/V3*A(3)-K34*A(3) + K43*A(4)
DADT(4) = K34*A(3)-K43*A(4)
DADT(5) = K25*A(2) - K52*A(5)
;FREE DRUG CONCENTRATION; 95% PB sunitinib, 90% PB SU12662
FC= A(3)/V3*(1-0.90)+A(2)/V2*(1-0.95)

; Hazard Model
DADT(6) = BLHAZ*EXP(BETA*FC)

\$ERROR

FREE = A(3)/V3*(1-0.90)+A(2)/V2*(1-0.95)

```
CHZ = A(6)
```

```
SUR = EXP(-CHZ)
HAZNOW = BLHAZ*EXP(BETA*FREE)
```

```
IF(DV.EQ.1) THEN
  Y = HAZNOW*SUR
ELSE
  Y = SUR
ENDIF
```

```
$THETA
(0, 0.00014) ; 1 TV_BLHAZ - BASELINE HAZARD
(-0.758) ; 2 TV_BETA - FACTOR
```

```
$OMEGA 0 FIX ; 1 ETA_HAZ
$OMEGA 0 FIX ; 2 ETA_BETA
```

```
$ESTIMATION SIG=2 SIGL=6 MAXEVAL=9999 METHOD=COND LAPLACE LIKE PRINT=1
```

```
$COVARIANCE PRINT=E
```
