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Dosage Optimization of Treatments Using Population Pharmacokinetic Modeling and Simulation

M. Guidi[ab], Mona Arab-Alameddine[ab], Margalida Rotger[c], Manel Aouri[b], Amalio Telenti[c], Laurent A. Decosterd[a], Thierry Buclin[a], Chantal Csajka[ab]*, and the Swiss HIV Cohort Study

Abstract: Pharmacokinetic variability in drug levels represent for some drugs a major determinant of treatment success, since sub-therapeutic concentrations might lead to toxic reactions, treatment discontinuation or inefficacy. This is true for most antiretroviral drugs, which exhibit high inter-patient variability in their pharmacokinetics that has been partially explained by some genetic and non-genetic factors. The population pharmacokinetic approach represents a very useful tool for the description of the dose-concentration relationship, the quantification of variability in the target population of patients and the identification of influencing factors. It can thus be used to make predictions and dosage adjustment optimization based on Bayesian therapeutic drug monitoring (TDM). This approach has been used to characterize the pharmacokinetics of nevirapine (NVP) in 137 HIV-positive patients followed within the frame of a TDM program. Among tested covariates, body weight, co-administration of a cytochrome (CYP) 3A4 inducer or boosted atazanavir as well as elevated aspartate transaminases showed an effect on NVP elimination. In addition, genetic polymorphism in the CYP2B6 was associated with reduced NVP clearance. Altogether, these factors could explain 26% in NVP variability. Model-based simulations were used to compare the adequacy of different dosage regimens in relation to the therapeutic target associated with treatment efficacy. In conclusion, the population approach is very useful to characterize the pharmacokinetic profile of drugs in a population of interest. The quantification and the identification of the sources of variability is a rational approach to making optimal dosage decision for certain drugs administered chronically.

Keywords: HIV · Nevirapine · NONMEM · Pharmacokinetics · Population

Population Pharmacokinetic and Pharmacodynamics Analyses

Drugs with a narrow therapeutic index, important inter-patient and low intra-patient variability, for which a good correlation between drug concentrations and markers of therapeutic success or toxicity has been shown, represent good candidates for therapeutic drug monitoring (TDM). This approach has long been recognized as a very useful tool to bring drug levels into a pre-specified target associated with optimal treatment success. Variability in drug levels under standard dosage regimen is considered as a major determinant of drug response, since it can lead to ineffective drug concentrations or toxic reactions. Of the numerous sources of variability, demographics, environmental, physiopathological factors and more recently genetic polymorphisms have been able to explain part of this variation.

In that respect, population pharmacokinetic and pharmacodynamic modelling has been recognized as an essential component for accurate description of dose-concentration-effect/toxicity relationships, quantification and explanation of variability in drug concentrations and effects in the target population of patients receiving a drug. Such techniques are ideally suited to describe the distribution of concentration values among patients and to define the target drug exposure to be reached in relation to drug efficacy and toxicity. This approach is a prerequisite for Bayesian treatment individualization, which is of particular importance when potent drugs with a narrow therapeutic index must be administered on the long term.

This approach has been shown to be useful for dosage optimization in the field of HIV therapies, for new targeted oncologic treatment, immunosuppression in transplant recipients, or psychoactive drugs, for which adequate characterization of the concentration-response surface and quantification of variability is of critical importance to improve therapy success while minimizing toxicity. As an example, a population pharmacokinetic study of nevirapine in the treatment of HIV infection is presented.

Variability in Nevirapine Exposure in HIV Positive Patients and in Relation to Treatment Success: An Example of a Population Pharmacokinetic Analysis

1. Introduction

Nevirapine (NVP) was the first non-nucleoside reverse transcriptase inhibitor (NNRTI) to be licensed for clinical use for the treatment of human immunodeficiency virus type 1 (HIV-1) infection. Due to its low cost, NVP remains one of the most widely prescribed antiretroviral drugs in resource-limited countries. Its pharmacokinetic (PK) profile is characterized by a rapid and almost complete oral absorption and a prolonged disposition phase.[1–4] NVP

*Correspondence: Dr. C. Csajka[ab]
Tel: +41 21 314 42 63
Fax: +41 21 314 42 66
E-mail: Chantal.Csajka@chuv.ch
[a]Department of Pharmaceutical Sciences
University of Geneva, Geneva
[b]Division of Clinical Pharmacology and Toxicology
University Hospital and University of Lausanne
Lausanne
[c]Institute of Microbiology
University Hospital and University of Lausanne
Lausanne

is mainly metabolized by CYP2B6 and, to a lesser extent by CYP3A4.[2,5–7] As for other antiretroviral drugs, nevirapine exhibits wide inter-patient variability in its pharmacokinetics, leading to sub-therapeutic or toxic levels under standard dosage regimens in a fraction of individuals. Several factors including genetic and non-genetic influences have been reported to affect NVP drug concentrations.[8–16]

NVP is used at the recommended dosage regimen of 200 mg twice daily (BID).[5] However, it has been shown that long-term suppression of viral load in HIV-positive patients requires their thorough adherence to therapy, which is easier to obtain with once-daily (QD) dosage regimens.[17–20]

The objective of this observational study was to quantify the pharmacokinetics of NVP in a large cohort of HIV-positive individuals, and to identify factors that might explain variations in drug levels. Co-administered drugs, demographic, clinical characteristics and genetic polymorphisms of *CYP3A4* and *CYP2B6* were tested as potential influencing factors. Simulations for 400 mg QD and 200 mg BID were performed in order to evaluate the percentage of patients with concentration over the recommended minimum target level of 3000 ng/mL associated with treatment success.[5]

2. Materials and Methods

2.1 *Study Population*

A total of 371 patients followed within the frame of a routine TDM program between March 2002 and May 2010 were included. The ethics committees approved the project and all participants gave written informed consent for genetic testing. NVP was administered at doses ranging from 100 mg to 600 mg, either QD or BID. A median of one concentration (range 1–14) was collected per patient.

2.2 *Genotyping and Analytical Methods*

Blood samples were collected into lithium heparin or EDTA-K Monovette syringes. Plasma was isolated by centrifugation, inactivated for virus at 60 °C for 60 min, and stored at –20 °C until analysis. Plasma NVP levels were determined by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using an adaptation of the methodology developed in our laboratory.[21] The calibration curves are linear with a lower limit of quantification of 250 ng/mL. Genotyping was done according to a previously published method.[10] Alleles are designated in concordance with the *CYP* Allele Nomenclature Committee (*http://www.xypalleles.ki.se*).

2.3 *Model-based Pharmacokinetic Analysis*

The non-linear mixed effects modeling program (NONMEM version VII, NM-TRAN v II[22]) was used. This approach allows estimating the population mean of the pharmacokinetic parameters with inter- and intra-individual variability and the influence of available factors on the estimates. Mono- vs. multiple-disposition models were compared. Exponential errors following a lognormal distribution were assumed for the description of the inter-patient and intra-patient variability.

Potential covariates influencing the kinetic parameters were incorporated sequentially in the model using linear models for continuous variables or coded as 0 or 1 for categorical covariates; a boundary condition of 1.5 times the upper limit of normal (ULN) was used to recode liver function tests into dichotomous variables. The baseline covariates evaluated were: gender, race, age, body weight, height, liver function tests (alanine amino transferases ALT and aspartate transferases AST) as markers of decreased hepatic function, chronic hepatitis C (HCV) infection and co-medications (classified as inducers or inhibitors of CYP3A4). For a subset of patients, genetic polymorphisms in *CYP3A4 *1B* and *CYP2B6* (**6*, **15*, **18* and **22*), associated with a decrease/loss in enzymes activity were tested for their impact on NVP clearance CL. Patients were categorized into genotypic groups according to the number of functional alleles, depending if they were carriers of 2 (reference allele; Hom-Ref, fully functional), 1 (heterozygote mutated; Het-LOF, partially functional) or unfunctional allele (homozygote mutated; Hom-LOF) for CYP2B6 **6*, **15*, *18; individuals carrying a gain of function allele (GOF = increased enzyme activity) for *CYP2B6*22* were given a score of 3.[23] The influence of genetic polymorphism on NVP CL was evaluated by a rich model that associates a separate fixed effect to each of the genotypic group (GOF, Ref, Het-LOF, Hom-LOF) (Eqn. (1)), as follows:

$$CL = CL_0 I_0 + CL_1 I_1 + CL_2 I_2 + CL_3 I_3 \quad (1)$$

where CL_0, CL_1, CL_2 and CL_3 are the typical value of CL for the Hom-LOF, Het-LOF, Hom-Ref and GOF groups and I_i is an indicator variable taking the value of 1 if an individual carries the *i*th genotype, 0 otherwise. Models relating CL with functional scores (0, 1, 2 or 3 depending on how many alleles were functional) were also tested and compared with the richest possible one (Eqn. (1)) using linear, power and square root functions of the activity scores, expressed by the equations:

$$CL = CL_0 + \theta_1 \times n \quad (2)$$

$$CL = CL_0 \times \theta_1^n \quad (3)$$

$$CL = CL_0 + \theta_1 \times \sqrt{n} \quad (4)$$

where n = 0, 1, 2 and 3 represents the functional score and θ_1 the average increase per active allele above CL_0, which is CL associated with fully decrease/loss enzyme activity (Hom-LOF).

2.4 *Parameter Estimation and Model Selection*

The data were fitted by use of the first-order conditional method. As a goodness-of-fit statistics, NONMEM® uses an objective function value (OF), which corresponds approximately to –2 log likelihood of the data. The selection between two models is based on graphical diagnostics and on a change in the OF (ΔOF), which approximates a χ^2 distribution. A decrease of the objective function is thus considered statistically significant ($P < 0.05$) if it exceeds 3.8 for one additional parameter.

2.5 *Model Validation and Simulations*

Model validation was performed by bootstrap resampling method using PsN-Toolkit (v 3.2.4).[24] Two hundred data sets were reconstructed by re-sampling from the original data. The mean values of the parameters obtained were compared with those estimated from the original data. In addition, the final model with variability was used to simulate 1,000 individuals and to calculate the average concentrations time profile with 95% prediction intervals under 200 mg BID and 400 mg QD. Concentrations at the end of the dosing intervals (C_{min}) were derived in order to compare obtained values with the threshold of 3'000 ng/ml, which is considered as the value to be targeted for treatment efficacy.[5] Comparisons of average C_{min} were performed using a Student t-test. The figures were generated with GraphPad Prism (V 5.0).

3. Results

3.1 *Data*

A total of 734 plasma concentrations were included in the population analysis. Measured concentrations ranged between 1065 and 22 040 ng/ml. A summary of the study population is presented in Table 1.

3.2 *Structural Model*

A one-compartment model with first-order absorption from the gastrointestinal tract was found to describe the data

Table 1. Characteristics of 371 model-building patients evaluated in the population pharmacokinetics analysis of NVP

Baseline characteristic	Model-building patients	
	Value	% or range
Demographic characteristics		
Sex (men/women) (no.)	241/130	65/35
Median age (yr)	48	24–82
Median body weight (kg)	69	40–125
Median height (cm)	172	145–195
Ethnicity (no. of patients)		
Caucasian	305	82
African	51	14
Asian	10	3
Hispanic	5	1
Clinical Chemistry (>1.5*ULN [a])		
ALT[a] (no.)	78	20
AST[a] (no.)	38	10
Bilirubinemia (no.)	3	0.8
Concomitant medications[b]		
Protease inhibitors		
Atazanavir and/or Ritonavir	63	23
Amprenavir	15	4
Saquinavir	13	3
Nelfinavir	12	3
Lopinavir	77	20
Reverse transcriptase inhibitors		
Lamivudine	200	53
Zidovudine	31	9
Didanosine	67	18
Tenofovir	155	41
CYP450 inducers		
Rifampicine (s)	2	0.5
Rifabutine	2	0.5
Carbamazepine (s)	2	0.5
CYP450 inhibitors		
Fluconazole	3	0.8
Fluvoxamine	1	0.2
Chronic Hepatitis		
HCV[a]	83	22
Genetic Polymorphisms[c]		
CYP 3A4 *1B (82 p)	4/6/72	5/7/88
CYP 2B6 *6 (114 p)	8/44/62	7/39/54
CYP 2B6 *15 (82 p)	0/1/81	0/1/99
CYP 2B6 *18 (82 p)	0/1/81	0/1/99
CYP 2B6 *22 (82 p)	0/5/77/0	0/6/94/0

[a]ULN, upper limit of normal; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV chronic hepatitis C. [b]Since a careful check of the data file revealed that a single patient can change his therapy with time, the values of the concomitant medications refer to the number of patients taking one of them at least once during the therapy. [c]The three values refer to the number of Hom-variants, Het-variants, Hom-Ref individuals and GOF for CYP2B6*22 characterized for each allelic variant. Het: heterozygous; Hom: homozygous; GOF: gain of function

appropriately. In addition to CL, the assignment of an interpatient variability on the volume of distribution V (ΔOF = –12.9 $p < 0.001$) improved significantly the fit, whereas no variability was observed on the absorption rate constant (k_a), probably due to the limited amount of data characterizing the absorption phase. The PK estimates and the variabilities (CVs) of the population model without any covariates were a CL (CV %) of 2.97 l/h (35%), a V of 104 l (58%) and a k_a of 1.06 h^{-1}.

3.3 *Non-genetic Covariate Analysis*

Univariate analyses performed using each available covariate identified that body weight (ΔOF = –18.1 $p < 0.0001$), height (ΔOF = –16.4 $p < 0.0001$), sex (ΔOF = –4.1 $p = 0.04$), AST (ΔOF = –9.3 $p = 0.002$), chronic hepatitis C (HCV) (ΔOF = –7.5 $p = 0.006$) co-administration of boosted atazanavir (ATV/RTV)(ΔOF = –16.0 $p < 0.0001$) and *CYP3A4* inducers (ΔOF = –10.5 $p = 0.001$) had a significant impact on NVP CL. Multivariate analyses showed that height and sex were correlated to body weight and chronic hepatitis C was correlated to AST. The final model included body weight, AST, ATV/RTV, and *CYP3A4* inducers (ΔOF = –49.5 $p < 0.0001$).

3.4 *Pharmacogenetic Analysis*

Genetic polymorphism in both *CYP2B6*6* and *CYP3A4*1B* significantly influenced NVP CL in the univariate analyses (ΔOF = –22.4 $p = 0.000002$ and –8.7 $p = 0.003$, respectively). The rich model estimated average clearances of 3.37, 2.82 and 1.85 l/h in carriers of the *CYP2B6*6* Hom-Ref, Het-LOF and Hom-LOF genotypes, respectively. Models relating CL with the number of functional alleles indicated that the square root function of the number of functional alleles (Eqn. (4)) best described the relationship between NPV CL and *CYP2B*6*. A similar trend was observed for the *CYP3A4*1B* alleles, yielding a reduction from 3.05 l/h in Hom-Ref to 2.67 and 1.86 l/h in Het-LOF and Hom-LOF individuals, respectively. Multivariate analyses revealed that solely *CYP2B6*6* allelic polymorphism remained statistically significant, in addition to other non-genetic covariates.

The final covariate analysis suggests that CL is increased by 29% (CI 95% 6–53%) on body weight doubling and increased by 36% (CI 95% 20–93%) in case of co-administration of a CYP3A4 inducer. Simultaneous administration of ATV/RTV and elevated AST on the other hand, reduced CL by 16% (CI 95% 5–26%) and 29% (CI 95% 14–44%), respectively. Individuals carrying 1 or 2 loss of function of the CYP2B6*6 alleles have a decrease of 16 % (CI 95% 5–27%) and 45 % (CI 95% 32–58%) resp. in NVP CL compared to individuals carrying the fully functional allele. The final model parameters' estimates, together with their bootstrap estimations, are summarized in Table 2. Fig. 1 shows the concentration-time plots of NVP after either 200 mg BID or 400 mg QD regimens, together with the 95% prediction intervals. Fig. 2 depicts the decrease in NPV CL associated with *CYP2B6*6* genetic polymorphism in the presence or not of an elevation of AST for a 70kg person, assuming no co-administration of a CYP3A4 inducer.

3.5 *Simulations of Dosage Regimens*

Simulations based on the final PK parameters without covariates predicted an average concentration at the end of the dosing interval (C_{min}) of 5204 ng/ml (95% PI: 1994–11545 ng/ml) for the 200 mg BID 12 hours after drug intake and of 4277 ng/ml (95% PI: 1231–9915 ng/ml) for the 400 mg QD dosage regimen 24 hours after drug intake. While taking into account

Table 2. Final population pharmacokinetic/pharmacogenetic parameter estimates of NVP and their bootstrap evaluations

Parameter	Population mean				Bootstrap evaluation			
	Estimate	SE[a] [%]	IIV[b] [%]	SE[c] [%]	Estimate	SE[a] [%]	IIV[b] [%]	SE[c] [%]
Model for the entire population n = 371								
CL (l/h)	3.05	2	32	36	3.06	2	32	35
θ_{BW}	0.39	25			0.40	24		
$\theta_{ATV/RTV}$	−0.16	29			−0.16	28		
θ_{INDC}	0.36	79			0.31	77		
θ_{AST}	−0.13	38			−0.13	38		
V (l)	95.9	11	56	53	96.3	12	56	55
k_a (h^{-1})	0.99	21			1.00	22		
σ (CV%)[d]	26	31[c]			25	32[c]		
Model for the subpopulation characterized for CYP2B6*6 genetic polymorphisms n = 114								
CL (l/h)[e]	1.81	10	26	58	1.82	12	24	65
$\theta_{CYP2B6*6}$[f]	1.07	14			1.06	17		
θ_{BW}	0.29	41			0.29	44		
$\theta_{ATV/RTV}$	−0.16	34			−0.16	42		
θ_{INDC}[g]	0.36	–			0.36	–		
θ_{AST}	−0.29	26			−0.27	32		
V (l)	80.4	22	58	71	82.4	30	62	79
k_a (h^{-1})	0.79	32			0.83	44		
σ (CV%)[d]	22	43[c]			22	46[c]		

CL, mean apparent clearance; V, mean apparent volume of distribution; k_a, mean absorption rate constant; BW, body weight; ATV/RTV, administration of atazanavir and ritonavir; INDC, *CYP3A4* inducers; AST, aspartate aminotransferase. [a]Standard errors of the estimates (SE) are defined as SE/estimate and are expressed as percentages. [b]Interindividual variability defined as CVs [%]. [c]Standard errors of the coefficient of variations, calculated as (SE/estimate)^0.5, are expressed as percentages. [d]Residual intrapatient variability, expressed as a CV [%]. [e]CL for the *CYP2B6*6* Hom-LOF patients. [f]Contribution of *CYP2B6*6* to NVP CL multiplied by $\sqrt{n}$ with n = 0, 1, 2 for Hom-LOF, Het-LOF and Hom-Ref patients respectively. [g]Fixed to the estimates obtained in the whole population.

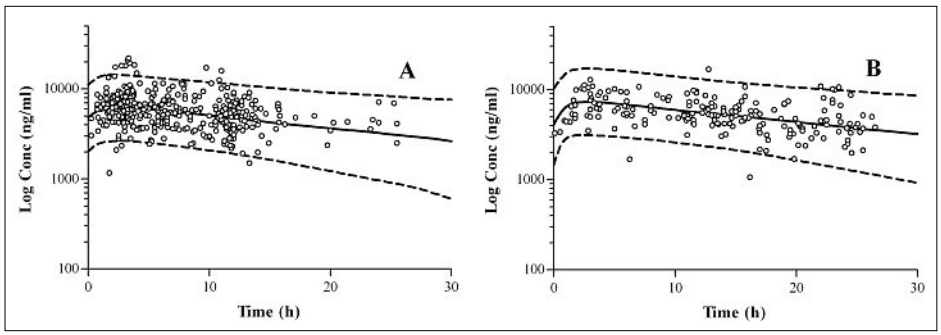

Fig. 1. Plasma NVP concentration-time plots of patients receiving 200 mg BID (A) and 400 mg QD (B) dose of NVP. Circles represent patient samples; solid line, average population prediction value; dashed lines, 95% prediction intervals.

the variability in the PK, 16% of patients after 200 mg BID are expected to have a C_{min} under the recommended minimum target level of 3000 ng/ml, whereas 30% would be below this target after 400 mg QD. Average C_{min} was 8092 ng/ml (95% PI: 2800–17974 ng/ml) after 400 mg QD in individuals with no functional *CYP2B6*6* allele, with only 3% of individuals with C_{min} below the target level of 3000 ng/ml.

4. Discussion

NVP is characterized by high inter-individual variability, of which 26% could be explained by body weight, co-administration of CYP3A4 inducers and inhibitors, elevated AST level and *CYP2B6*6* polymorphisms. The presence of such factors might lead to sub-therapeutic drug levels in some individuals and are thus important to be identified in order to optimize drug regimens. PK estimates are in good accordance with previously published reports.[8,9,12,14,15] Body weight has been shown to be associated with NVP CL in several studies.[8,12,15] As expected and previously reported, concomitant administration of strong CYP3A4 inducers affects NVP exposure.[25] Although co-administration of ATV/RTV with NVP is not recommended in antiretroviral therapy,[26] 17% of our study participants were taking both drugs. A small 16% reduction in NVP elimination was observed, which is of limited clinical relevance with regard to NVP. Similarly to our results, elevated AST level has been previously reported to modestly decrease NPV CL.[8] It remains however unclear whether the elevation of hepatic enzymes is the cause or the consequence of high NVP levels, since NVP is a hepatotoxic drug. Among all tested variables, genetic polymorphism in *CYP2B6*6* had the most profound impact on NVP elimination.[10,11,13–16] In our population, it explained 13% of NVP variability. As already described for efavirenz,[23] the use of an activity score associated with the number of functional alleles could describe the influence of this genetic polymorphism using a square root function, suggesting that the enzyme activity was almost totally maintained in carriers of one functional allele, whereas it was markedly reduced in carriers of the homozygote mutation. This phenomenon suggests adaptive mechanisms, possibly through the activation of nuclear receptors.[27]

Several studies have compared the

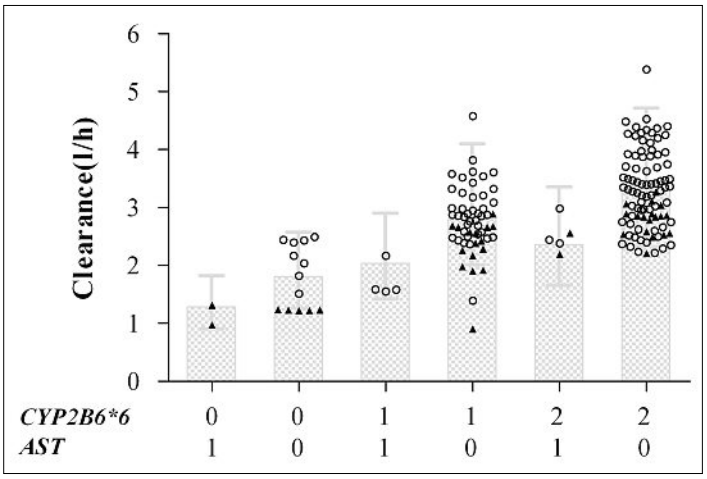

Fig. 2. Individual (open circles) and average predicted clearences (bars) with 90% population prediction intervals for each combination of elevated aspartate aminotransferase (AST) (coded as 1 when AST > 1.5*upper limit of normal, 0 otherwise) and *CYP2B6*6* functional alleles (coded as 2 for homozygote reference alleles, 1 for heterozygote mutated and 0 for homozygote mutated alleles, for a 70 kg person. The triangles represent CL associated to patients taking ATV/RTV in addition to the other depicted covariates.

once- and the twice-daily nevirapine immediate-release dosage regimens in order to identify the dose that optimizes efficacy, while improving adherence and minimizing toxicity. Despite the absence of a clear relationship between drug exposure and toxicity,[28–33] some evidence suggests that the 400 mg QD regimen might be associated with an increased risk of hypersensitivity reactions leading to treatment interruption.[33,34] Our simulations indicate that concentration exposure is less frequently maintained over the target trough level with the 400 mg QD regimen than with the currently recommended 200 mg BID regimen. However, in the subpopulation of individuals carrying the mutations in the CYP2B6*6 genotype, the once-daily therapeutic scheme performs better, but might be related to an increased risk of toxicity.

In conclusion, NVP PK is influenced by several environmental and physiopathological factors and by *CYP2B6*6* genetic polymorphisms that should be accounted for in dosage individualization. Simulations suggest that the 400 mg once daily dosage regimen would provide sub-optimal drug levels according to target concentrations in a higher number of patients than the 200 mg twice-daily regimen. The recent approval by the FDA of an extended-release formulation has been shown to circumvent this issue. Dose adjustment in a sub-population of patients showing high exposure in relation with genetic traits may be considered as a dosing strategy with the potential to reduce both costs and toxicity.

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