EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

12 December 2011
EMA/CHMP/37646/2009
Committee for Medicinal Products for Human Use (CHMP)

Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products

Draft Agreed by Pharmacogenomics Working Party and EWP- PK	March 2010
Adoption by CHMP for release for consultation	22 April 2010
End of consultation (deadline for comments)	31 October 2010
Agreed by Pharmacogenomics Working Party	October 2011
Adoption by CHMP	19 January 2012
Date for coming into effect	1 August 2012

This guideline replaces the Reflection Paper on the use of Pharmacogenetics in the Pharmacokinetic Evaluation of Medicinal Products (EMEA/128517/2006).

Keywords	*Pharmacogenetics, pharmacokinetics, clinical development*

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
Email info@ema.europa.eu **Website** www.ema.europa.eu

Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products

Table of contents

Executive summary

This guideline addresses the influence of pharmacogenetics on drug pharmacokinetics, encompassing considerations and requirements for the design and conduct of investigations during drug development. For those cases where pharmacogenetics is envisioned to play a major role in the benefit-risk of a medicinal product because of its impact on pharmacokinetics, guidance is given regarding studies required and recommended at different phases of drug development to ensure satisfactory efficacy and safety in genetic subpopulations that have variable systemic exposure of active substances.

1. Introduction

The pharmacokinetics of many medicinal products is prone to interindividual variability, which is caused by several factors such as gender, age, weight, impaired renal and hepatic function, and genetics. In recent years, a rapid development in our understanding of the influence of genes on interindividual differences in drug action has occurred. This development encompasses the area of pharmacogenomics, including pharmacogenetics. It is acknowledged that pharmacogenetics may not be equally important for every drug. However, for drugs where pharmacogenetics is important for pharmacokinetic variability, this Guideline provides a framework on where it is recommended that pharmacogenetics should be implemented in the drug development process. Details on conditions where further investigations are warranted are provided in the body of this Guideline.

Background

In the field of pharmacogenetics, interindividual variability in genes influencing or predicting the outcome of drug treatment (e.g., genes encoding drug transporters, drug metabolising enzymes, drug targets, biomarker genes) is studied in relation to efficacy of drug treatment and adverse drug reactions. Some of this interindividual variability is caused by genetic variation, i.e., the occurrence in the same population of multiple allelic states. Examples of genetic variations include Single Nucleotide Polymorphisms (SNPs), insertions/deletions and variation in gene or sequence copy number (copy number variation, CNV).

Our main knowledge on genetic factors influencing absorption, distribution, metabolism and excretion (ADME) is centred on drug metabolism. Genetic variations in metabolizing enzymes may lead to (i) increased or decreased clearance of the parent drug or pharmacologically active or toxic metabolites, (ii) increased or decreased production of active metabolites of the respective prodrugs, or (iii) increased or decreased formation of toxic products. These metabolising steps may involve phase I and/or phase II enzymes.

The normal (wild-type) situation with a certain metabolising capacity, is referred to as 'extensive metabolisers' (EM). Increased metabolism occurs in the 'ultrarapid metaboliser' (UM), and is usually the result of multiple active alleles; decreased metabolism occurs in the 'poor metaboliser' (PM), and is usually the result of mutations or gene deletions leading to reduced or abolished expression or function of the respective enzymes. 30-50% of all clinically used drugs are metabolized by functionally polymorphic enzymes [1,2], including Phase I cytochrome P450 enzymes (e.g., CYP2C9, CYP2C19 and CYP2D6[3]), and phase II enzymes (e.g., UDP-glucuronosyltransferases, *N*-acetyltransferase-2 and some methyltransferases).

Metabolising enzymes account for 80% of the genes/enzymes that are mentioned for pharmacogenetic purpose in the current drug labels[4]. Examples of polymorphisms affecting the benefit-risk of medicinal products in subpopulations of patients are known. For example:

(i) For many antidepressants and antipsychotics, which are known CYP2D6 substrates, the plasma levels of the drug at the same dosage often vary 5-20-fold, an important factor being polymorphisms in the CYP2D6 enzyme. There are many reports of increased frequency of adverse drug reactions among subjects with the poor metaboliser phenotype, due to increased systemic exposure to the parent drug[5]. Furthermore, exposure to some important anticoagulants e.g., warfarin and acenocoumarol is dependent on the CYP2C9 genotype of the patient[6,7].

(ii) Excessive prodrug activation may affect safety of codeine (CYP2D6), tramadol (CYP2D6), and clopidogrel (CYP2C19). Hence, ultrarapid metabolisers suffer from adverse events due to increased levels of active metabolites. The efficacy of prodrugs which are activated by polymorphic enzymes may also vary depending on the presence of specific functional allelic variants in patients. An example of this is clopidogrel, for which the conversion of the clopidogrel prodrug to active drug is much diminished in about 20% of Asian patients being CYP2C19 poor metaboliser, and this reduced metabolism results in less anti-coagulation and less protection against cardiovascular events[8,9]. The latter example also illustrates that pharmacogenetically based variation in pharmacokinetics may subsequently be important for pharmacodynamics and benefit-risk considerations.

It is important to mention that in these examples the consequences of genetic polymorphism were noted after registration of the medicinal product. However, in the future it is anticipated that the possibility that enzyme polymorphism leads to a different benefit-risk in certain genetic subpopulations[i] is considered prior to registration, and this Guideline aims to provide a framework for doing so.

In recent years, journal articles have been published describing specific polymorphisms in drug transporters and their possible effect on the efficacy and safety of medicinal products. However, in the majority of cases the influence of transporter polymorphism on drug pharmacokinetics has not yet been clarified. One exception is the SLCO1B1 (OATP1B1) polymorphism which has been shown to significantly affect the pharmacokinetics and adverse effects of some drugs, mainly statins[10,11,12]. However, in general, the effect of transporter polymorphism on drug pharmacokinetics has not been extensively evaluated, compared with polymorphic phase I and phase II metabolising enzymes. Importantly, transporter polymorphism may not only affect systemic exposure, but also or only local (target) exposure, which is more complex to monitor. It is anticipated that more examples will be described in this area as the research continues, and the possibility of transporter polymorphism as a cause of altered pharmacokinetics must always be considered during drug development.

Until now, it has been difficult to transfer knowledge of the effect of polymorphism into specific recommendations in affected genetic subpopulations[ii]. In this respect, genetic subpopulations have been treated differently than other subpopulations or circumstances in which the exposure of active or toxic substances is decreased or increased, like in case of renal or hepatic impairment or in case of drug-drug interactions. The aim of including pharmacokinetics-related pharmacogenetics in drug development is to evaluate whether exposure in genetic subpopulations is different to such an extent that this would require a change in the posology or treatment recommendation of the drug for the specific subpopulation.

[i] The term “genetic subpopulation” may include both the phenotype, e.g. poor metaboliser, as well as the genotype, e.g., CYP2D6*4.
[ii] The term “genetic subpopulation” may include both the phenotype, e.g. poor metaboliser, as well as the genotype, e.g., CYP2D6*4.

2. Scope

The aim of this guideline is to clarify the requirements related to the use of pharmacogenetics in the pharmacokinetic evaluation of medicinal products. This guideline applies predominantly to small molecule drugs as genetic effects on the pharmacokinetics of biological drugs today are much less understood.

The following issues are discussed in this guideline:

- In which situations and at what stage(s) in the clinical development program should pharmacogenetics related pharmacokinetic studies be performed.
- Recommendations or requirements regarding pharmacogenetics related pharmacokinetic studies investigating the effects of polymorphisms at the ADME level (enzymes, transporters, binding proteins and other relevant proteins), including study design, selection of subjects, and sampling.
- Evaluation of the clinical impact of genetic differences on pharmacokinetic parameters and recommendations on further studies to support the posology/treatment recommendations for genetic subpopulations.
- Possible consequences of genetically determined differences in pharmacokinetic parameters for treatment recommendations and labelling.
- Special considerations related to drug-drug interactions as it relates to pharmacogenetics related pharmacokinetic studies.
- The effect of impaired or immature organ functions as it relates to pharmacogenetics related pharmacokinetic studies.

3. Legal basis

This guideline applies to Marketing Authorisation Applications for new medicines for human use submitted in accordance with Article 8(3) of the Directive 2001/83/EC, as amended. This guideline should be read in conjunction with the Introduction and general principles paragraph (4) and Part I of the Annex I to Directive 2001/83, as amended, and all other relevant information included in current and future EU and ICH guidelines and regulations especially:

- Note for Guidance on Good Clinical Practice - CPMP/ICH/135/95 (ICH E6).
- Note for Guidance on General Considerations for Clinical Trials - CPMP/ICH/291/95 (ICH E8).
- Pharmacokinetic studies in man - EudraLex vol. 3C C3A.
- Guideline on reporting the results of population pharmacokinetic analyses - CHMP/EWP/185990/06.
- Note for Guidance on the investigation of pharmacokinetic drug interactions - CPMP/EWP/560/95.
- Guideline on the investigation of bioequivalence - CPMP/EWP/QWP/1401/98.
- Guideline on the role of pharmacokinetics in the development of medicinal products in the paediatric population - EMEA/CHMP/EWP/147013/2004.
- Guideline on the evaluation of the pharmacokinetics of medicinal products in patients with impaired hepatic function - CPMP/EWP/2339/02.

- Note for guidance on the evaluation of the pharmacokinetics of medicinal products in patients with impaired renal function - CHMP/EWP/225/02.
- Position paper on terminology in Pharmacogenetics - EMEA/CPMP/3070/01.
- Rules governing medicinal products in the European Union Volume 2C Notice to applicants; A guideline on summary of product characteristics (SmPC) September 2009.
- Note for Guidance on definitions for Genomic biomarkers, pharmacogenomics, pharmacogenetics, genomic data and sample coding categories - EMEA/CHMP/ICH/437986/2006 (ICH Topic E15.).
- Note for Guidance on genomic biomarkers related to drug response: context, structure and format of qualification submissions - EMEA/CHMP/ICH/380636/2009 (ICH Topic E16).

4. Situations and stage in development where the effect of pharmacogenetics on pharmacokinetics should be considered

4.1. General recommendations

Genetic variants can influence drug pharmacodynamics but also the absorption, distribution, metabolism and excretion of a drug. Furthermore, pharmacogenetics may also influence the formation, distribution and elimination of metabolites and this should be remembered if there are metabolites that may affect the efficacy and/or safety of the administered drug. Genotypes leading to absent, decreased or increased enzyme or transport protein activity affecting the pharmacokinetics of the investigated drug and major pharmacologically active metabolites should be considered.

Studies of the effect of pharmacogenetics on the pharmacokinetics of an active substance (parent and/or active metabolites) and its implications for efficacy and safety during development are generally **required** when the magnitude of the interindividual variation in drug exposure is so high as to likely influence the safety and/or efficacy of the drug in genetically variable populations. Factors that identify such a situation are:

a) *in vitro* and/or *in vivo* studies indicate that a known functionally polymorphic enzyme or transporter is likely to represent an important pathway in the metabolism or distribution of the drug, *or*

b) *in vitro* and/or *in vivo* studies indicate that a known functionally polymorphic enzyme or transporter is likely to represent an important pathway in the formation, elimination or distribution of a pharmacologically active or toxic metabolite, or

c) *in vivo* studies indicate substantial interindividual differences in the pharmacokinetics of the drug *likely* to influence the efficacy or safety of the drug in the variable subpopulation, which can not be explained by other intrinsic or extrinsic factors.

Studies on the effect of pharmacogenetics on the pharmacokinetics of an active substance and its implications for efficacy and safety are generally **recommended** during development if:

d) available *in vitro* data indicate that a human polymorphic enzyme or transporter contributes to the pharmacokinetics of the active substances but the quantitative role may be low based on the *in vitro* data, *or*

e) there is high interindividual pharmacokinetic variability, or there are pharmacokinetic outliers with higher or lower exposure to the active substances, which cannot be attributed to other

known intrinsic or extrinsic factors, but which *possibly* can give rise to clinical efficacy and safety concerns based on the existing knowledge, *or*

f) major differences in pharmacokinetics are observed in different ethnic groups, which cannot be attributed to other known intrinsic or extrinsic factors.

Cut-off values defining 'important pathway' for decision making purpose in the above described situations are provided section 4.2.

In case important pharmacokinetic variability that is likely to influence clinical or safety aspects cannot be explained by non-genetic intrinsic or extrinsic factors, analysis of genes potentially responsible for the variation (such as phenotype-genotype associations) should be carried out. If e.g., candidate gene and targeted ADME SNP analyses do not offer an explanation for the pharmacokinetic variability observed, more thorough investigations are recommended when appropriate to understand the genetic contribution to variability in exposure (see sections 5.2 and 5.3).

If important interindividual variability in drug pharmacokinetics is observed but no apparent genetic polymorphism has been identified which can predict the pharmacokinetic outliers, phenotyping offers an alternative approach if reproducible data can be achieved at safe levels of the drug in the outlier population. Thus, dose adjustment in further phases of the developmental programme can be based on phenotyped individuals.

Special attention must be paid to specific outliers where an important pharmacokinetic alteration might be caused by a rare but functionally very important gene variant. In such a case a pro-active analyses of all possibly relevant genes is recommended.

If a polymorphism has been shown not to affect the functional performance or expression of the protein during *in vitro* or *in vivo* studies, genotyping for this polymorphism[iii] is not considered to be necessary during the clinical development program. The same is true if the results of pharmacokinetic studies clearly show that the impact of pharmacogenetics is not clinically relevant based on pre-specified, well supported target exposure limits.

Interindividual differences in pharmacodynamics may be a result of pharmacogenetically based variation in pharmacokinetics. Thus, in case important interindividual differences in pharmacodynamics is observed in the clinical trials, the possibility of polymorphic enzymes being involved resulting in difference in pharmacokinetics must be considered, including the evaluation of e.g. additional metabolic pathways not previously studied.

Still, in all clinical phases of development, prospective banking of DNA for genotype analyses is highly recommended, even when there are no obvious indications of a relevant genetic influence on pharmacokinetics. This recommendation is due to the fact that unknown polymorphic sites of importance can be identified later and that unknown but important metabolic or transport pathways in which a polymorphism might have an effect, may be identified at later stages in the development program or even post-marketing. This, e.g., has occurred for tamoxifen and clopidogrel, where activation by polymorphic enzymes has both been identified during pharmacovigilance monitoring[9,13].

4.2. Integrating pharmacogenetic effects on pharmacokinetics in drug development

In the following section, recommendations are made on how to implement pharmacogenetics during the different phases of clinical development, starting with the *in vitro* studies conducted before investigation of the medicinal product in man. In this section it is assumed, based on *in vitro*

[iii],When the term genotyping is used in this guideline, phenotyping by, for example, catalytic assays may also be an acceptable approach

information, that a known functionally polymorphic enzyme or protein is involved in metabolism or transport of the drug.

4.2.1. *In vitro* studies prior to human exposure.

Human *in vitro* metabolism studies are to be conducted prior to phase I (see also *Note for Guidance on the investigation of drug interactions* CPMP/EWP/560/95). Such studies preferably include identification of the enzymes catalysing the *in vitro* metabolism and also the identification and characterisation of metabolites formed through candidate major metabolic pathways, enabling early pharmacological activity screening of these metabolites. In case of an active parent drug, in the *in vitro* context for metabolising enzymes the following arbitrary rule is proposed: a pathway can be considered 'important' when based on *in vitro* data >50% of the drug is predicted to be cleared via a single polymorphic enzyme. Such a 50% reduction of clearance would give rise to a doubled exposure, which in an early PK study would probably be equal to increasing the dose to the next level. The aim is to avoid accidently exposure of poor metabolisers enrolled in an early study to non-studied exposures.

It should also be remembered that polymorphic enzymes can participate in the formation and elimination of pharmacologically active metabolites of the drug, including toxic metabolites.

Based on the *in vitro* data, the involvement of known functionally polymorphic enzymes in the metabolism of the parent compound and/or the formation and elimination of active metabolites can be predicted. As *in vitro* studies are not always quantitatively predictive of the *in vivo* situation, the enzyme involvement needs confirmation *in vivo*. However at this stage, the knowledge available should be used to find candidate enzymes involved in major drug metabolism pathways. For some enzyme systems, where well validated in silico Physiologically Based Pharmacokinetic (PBPK) models have been developed, these can be used to predict pharmacogenetic differences in human at this stage and to guide clinical study design with respect to pharmacogenetic investigation.

Involvement of transporters may also be indicated by *in vitro* data obtained prior to Phase I. The *in vivo* importance of a transporter may be implied through use of animal models, *in vitro* cellular systems, or information on similar substances. However, at the moment it may be difficult to make quantitative predictions of the *in vivo* contribution of transporters. For this reason, currently no cut-off level can be provided for transporters.

4.2.2. Phase I (exploratory)

4.2.2.1. First time in man studies

The possibility of genetic influence on the drug's pharmacokinetics should be considered early in the Phase I program. When the *in vitro* data indicate that a relevant involvement of a known functionally polymorphic enzyme cannot be excluded (i.e., in vitro data predict >50% to be cleared by a single polymorphic enzyme *in vivo*), it is advised to genotype the first time in man study population for the relevant genes in order to avoid safety issues related to genetically determined differences in active substance exposure. Subjects with a genotype predicted to result in markedly increased exposure of active substances should, preferably, only be allowed to enter in the first time in man study at doses several-fold lower than the doses expected to be safe in extensive metabolisers.

Presently, knowledge of transporter protein polymorphisms with exception for SLCO1B1 is not mature enough to estimate the potential for significant involvement of the transporter polymorphism *in vivo* based on *in vitro* data. Unless there are other indications of significant transporter polymorphism involvement *in vivo*, early genotyping for a transporter gene is not indicated on the basis of *in vitro* data only. Still, prospective storing of samples in order to allow eventual pharmacogenetic analysis is highly recommended.

If future knowledge of drug transporters expands to such extent that certain *in vitro* data on transporters may be considered predictive of the clinical situation, the same protocol as described for metabolising enzymes may also be appropriate for polymorphisms in drug transporter encoding genes.

4.2.2.2. Phase I (further exploration)

In Phase I, the relative contribution of the identified polymorphic enzyme on the *in vivo* pharmacokinetics of a drug or active metabolite is estimated. In addition, if known functionally polymorphic transporters such as certain OATPs are found to be of importance for the drug's clearance, the effect of genetic polymorphism should be investigated. If potential effects of transporter polymorphism on distribution are indicated, the inclusion of PD markers could be considered.

It is recommended, if feasible, to investigate this in a conventional pharmacokinetic study including genetically defined subpopulations. If this is not possible, but based on the scientific literature or own validation data, the effect of a genotype may be mirrored with confidence by treatment with an inhibitor of the protein, the effect of the polymorphism could be estimated using the results of an *in vivo* interaction study with such an inhibitor. PBPK simulations may also be used to estimate the effect of carrying a certain rare genotype if the simulation is sufficiently supported by *in vivo* data (see section 5.1 for requirements related to study design). If a marked effect (arbitrarily defined as a situation where >25% of the parent drug is cleared by the polymorphic enzyme) of polymorphism is confirmed *in vivo*, it is recommended, where relevant, to expand the clinical Phase I program and also evaluate relevant interactions, as well as the consequences of impaired/immature organ function in the genetic subpopulations (see sections 8.1 and 8.2). The 25% cut-off is in line with the cut-off applied in case of drug-drug interactions (see *Note for guidance on the investigation of drug interactions* CPMP/EWP/560/95). Furthermore, dose-proportionality in poor metabolisers at relevant doses may be different than in the general population, and this should be investigated (see section 5.1). This evaluation should preferably be done before starting Phase III, to allow taking the results of this evaluation into consideration in the Phase III study protocol.

When based on available *in vitro* or preliminary clinical data, the genotype is predicted or known to affect the pharmacokinetics of pharmacologically active compounds, i.e., active drug or active or toxic metabolites, to a possible clinically relevant extent genotyping for the indicated genes is required in as many of the Phase I studies as possible in order to increase the amount of data that will support the recommendations for use in the genetically defined subpopulation(s). This genotyping should be done when e.g., *in vitro* data predict >50% of the active parent drug is cleared by a single polymorphic enzyme *in vivo*, or when >25% is cleared *in vivo*. In case of active metabolites, situations triggering further genotyping of relevant genes are considered present when a polymorphic enzyme is responsible for >25% of the in vivo formation or elimination of an active metabolite which is estimated to contribute to >50% of the pharmacodynamic effect or efficacy.

Furthermore, if there is high interindividual pharmacokinetic variability observed, or there are pharmacokinetic outliers with higher or lower exposure to the active substances observed in initial clinical studies which *possibly* can give rise to clinical efficacy and safety concerns based on the existing knowledge, investigations aimed at identifying the causes (either non-genetic or genetic) are recommended.

4.2.3. Phase II (dose finding, exploratory)

If Phase I studies indicate that pharmacogenetics influences the pharmacokinetics of a drug to a possible clinically relevant extent (i.e., >25% of the drug is metabolised by a single polymorphic enzyme), this should be reflected in the design of the Phase II studies. In case no genotype/phenotype-based dosing is applied to normalise drug exposure (i.e., to bring drug exposure

into the target range), the exposure level obtained in the genotypically-defined subpopulation should be studied in the Phase II study. This can be done either by including a sufficient number of the genotypically-defined group of patients with deviating activity, or by adjusting the dose yielding the target exposure in patients, not carrying the genotype with altered protein activity.

If active substance exposure is not normalised through genotype- or phenotype-based dosing or dose titration, sufficient data need to be collected in Phase II and III on the consequences of the altered exposure on the clinical efficacy and safety of the drug (see section 7). For this purpose, pharmacodynamic data (usually target effects) as well as safety data should be collected.

If based on Phase II data, the difference in exposure observed between extensive and ultrarapid/poor metabolisers is likely to be of clinical importance, then intermediate metabolisers should be investigated in a pharmacokinetic study, or through PBPK simulations (see section 5.1).

The ultimate aim of the Phase II investigations should be to optimise dose(s) selection and design of the Phase III studies, including the choice on whether genotype-based dosing should be applied or no dose correction seems needed based on genotype.

4.2.4. Phase III (confirmatory)

If available data indicate that there is a significant difference in drug/metabolite exposure or distribution in the genetically/phenotypically defined subpopulation (i.e., scenarios a-c in section 4.1 apply), genotyping for the relevant genes in all patients included in phase III studies is required, or alternatively phenotyping e.g., using a safe dose of the drug, and subsequent measurement of the bioactive metabolite. If scenarios d-f in section 4.1 apply, genotyping for the relevant genes is recommended. In all these scenarios, banking of samples for possible future pharmacogenetic analysis is highly recommended. Depending on the likely consequences of the polymorphism for efficacy or safety and knowledge from earlier phases of development, there are several ways that this knowledge could influence the design of Phase III studies. The following possibilities are envisioned:

a) The available data up to Phase II suggest (but are insufficient to prove) that a marked difference in exposure lacks clinical relevance, and no genotype/phenotype-specific treatment is aimed for. In this situation the Phase III study should aim at confirming this presumed lack of clinical significance of the different exposure. The conclusion on comparable efficacy and safety obtained in the subjects having low or high exposure of the parent drug needs to be supported by conclusive clinical data obtained at these exposure levels. For this purpose, a sufficient number of the genetic subpopulations should be included in the Phase III study. In case of low prevalence of poor metabolisers, the inclusion of an additional treatment arm with increased exposure may be needed. PK-PD data related to efficacy and safety may be supportive in this respect.

b) The available data up to Phase II suggest that the difference in exposure is likely to be clinically relevant, and a genotype/phenotype based dosing regimen yielding comparable exposure is developed in Phase I/II. In this case the exposure of active substances in the Phase III study is normalised through genotype/phenotype-based dosing based on knowledge of the difference in exposure in carriers of certain alleles. Sparse sampling with population-pharmacokinetic analysis may be applied to confirm the exposure normalisation.

c) The available data up to Phase II indicate that the difference in exposure is likely to be clinically relevant, and dose titration regardless of genotype is pursued (in case a suitable marker exists). Then the Phase III study should aim at confirming that there are no efficacy and safety concerns for the genetic subpopulation if the proposed general dose titration is applied. PK-PD data related to efficacy and safety may be supportive in this respect.

d) The available data indicate that the difference in exposure is likely to be clinically relevant, but it is not possible to normalise the exposure with the formulations to be marketed. In this situation patients of a specific genotype/phenotype (i.e., patients at risk) should be excluded.

In case polymorphic transporters are concerned, exposure in plasma may not be different between the different genotypes, however, altered intracellular or interorgan distribution may occur. In this case the consequences depend on the relationships between local exposure and pharmacodynamics of the medicinal product. If indicated, genotyping for the relevant transporter genes in phase III clinical studies is encouraged to explore consequences of such genetic variations.

The final aim of the clinical development program should be to obtain a clear dosing or treatment recommendation, yielding effective and safe treatment in the genetic/phenotypical subpopulations.

4.3. Involvement of relevant polymorphic proteins identified in the course of the clinical development program

In Section 4.2 of this guideline the ideal situation is described, where the potential effect of pharmacogenetics is detected early in drug development and further investigated in the Phase I, II and III sequence within the clinical development program. In situations where knowledge on possible genetic effects on the pharmacokinetics of the drug is lacking when initiating the clinical part of the development program of a new medicinal product, acquired pharmacokinetic (e.g. high variability in exposure), clinical efficacy and safety information at a later stage may trigger the need for investigations of the pharmacogenetic impact on drug or metabolite exposure. This situation may occur, e.g.:

a) when a previously unknown or sparsely studied functionally polymorphic enzyme or transporter is found to be involved in the metabolism or transport of the medicinal product that is being developed,

b) if the enzyme or transporter involved in metabolism or transport is known but there was no prior knowledge regarding functional polymorphisms of the gene.

c) When pharmacokinetic outliers are observed in the course of Phase I to IV studies.

Population pharmacokinetic analysis may be used as a hypothesis-generating tool where the effect of new or unexpected polymorphisms may be indicated (see section 5.1). If relevant information becomes available during the clinical development program or during pharmacovigilance monitoring, the relative contribution of the metabolism or transport pathway in question to the bioavailability, distribution and/or metabolism of the medicinal product *in vitro* and/or *in vivo* should be estimated. Further required or recommended pharmacogenetic investigations to be initiated from this point depend on the conditions as indicated in section 4.1.

Meta-analysis can be considered on pooled data from different pharmacokinetic/clinical studies, in order to guide further drug development. Preferably the included studies should be similar with respect to non-genetic factors which may affect the pharmacokinetics of a drug.

Conclusions from a retrospective analysis carried out in response to emerging data may be acceptable for genetic issues related to pharmacokinetics if mechanistically supported by available *in vitro* or pharmacokinetic information. In this case, DNA should preferably be available from a large proportion of patients in the Phase I, II and III studies. If a new genetic association is discovered in a retrospective analysis, complementary studies, such as *in vitro* studies or pharmacokinetic studies investigating the mechanism and confirming pharmacokinetic consequences of this finding, will be expected as additional support.

In specific cases it may be appropriate to contact the European Medicines Agency to discuss the issue during a pharmacogenetic briefing meeting or a Scientific Advice meeting.

5. Study design and methodology

5.1. Conventional pharmacokinetic analysis and population pharmacokinetic analysis

The pharmacokinetic study that is preferred for investigation of the effect of polymorphism on the exposure of pharmacologically active substances is of conventional, frequent blood sampling, design. A Phase I study of reduced design, i.e., including the extremes of genotypes (e.g., extensive vs. poor metabolisers) is usually performed as a basis for the evaluation of the pharmacogenetic effect on active substance exposure. The study populations should as far as possible be matched for intrinsic factors that may affect the pharmacokinetics of the drug. *In silico* PBPK modelling and simulation may be helpful when optimising the design of *in vivo* pharmacokinetic studies. Consequences of being an intermediate metaboliser can be estimated by expanding the reduced design pharmacokinetic study, or through PBPK simulations. The intermediate metaboliser status may be shown either by genotyping or phenotyping.

The study may be of single-dose design. If an effect of genotype is observed under single-dose conditions, the possibility to extrapolate the effect to multiple dose conditions should be considered. If extrapolation cannot be performed, e.g., due to non-linear pharmacokinetics and time dependence, a multiple-dose (steady state) study is needed. Two different doses can be used to add information on the linearity in pharmacokinetics in the genetic subpopulation. However, evaluating two dose levels in ultra-rapid metabolisers is not necessary if the drug shows linear pharmacokinetics in wild-type gene carriers

The conventional pharmacokinetic study should, in principle, include enough subjects for a likely clinically relevant difference in exposure to be detected between the included genotypes. However, if homozygotes for the allele(s) giving rise either to the most marked effect on protein activity are difficult to recruit due to very small allele frequency (e.g. <1%), as many carriers of the rare extreme genotype as possible should be included together with a larger number of heterozygote carriers or gene variant carriers having an intermediate protein activity. This may allow a preliminary estimation of the consequences of this polymorphism in subjects who are homozygous for the variant. When a conventional pharmacokinetic study is not possible, and the effect of genotype is known or shown to be mirrored by treatment with an inhibitor of the protein, the use of a drug-drug interaction study with a extensively validated inhibitor may be considered (see section 4.2.2.2). For specific requirements related to drug-drug interaction studies, see the *Note for guidance on the investigation of drug interactions* CPMP/EWP/560/95. Data obtained in a drug-drug interaction study may also be used to support PBPK simulations of the effect of carrying a certain genotype. However, this is under the assumption that the model satisfactory predicts the following *in vivo* data: a) the effect of the inhibitor on the exposure of the investigational drug, b) the effect of the inhibitor on a probe drug for the inhibited protein, and c) the effect of genotype on a probe drug for the protein.

Population pharmacokinetic analysis may be used as a hypothesis-generating tool where the effect of new or unexpected polymorphisms may be indicated. If a need for genotype-based dose adjustments has been identified, this should generally be supported by data on the effect of genotype generated from a conventional pharmacokinetic study, as in such cases a precise estimation of the genotype effect is needed. Population pharmacokinetic analysis of sparse data from Phase III may be used as supportive data indicating that a genotype-based dosing or treatment recommendation applied in Phase III has normalised drug exposure in the patient population. The study population should include

a satisfactory number of patients of each genotype, and enough samples per patient to obtain valid estimates.

5.2. Genotyping methods

No definite guidance with respect to the choice of the method determination of the genetic polymorphism can be given. At present, a rapid development is taking place with respect to analytical methods available for allele specific genotyping, such as real time polymerase chain reaction (RT-PCR), SNP/CNV arrays, combined mass-spectrometry, pyrosequencing, genomic sequencing, next generation sequencing etc at a decreasing cost. It is anticipated that the next generation sequencing methods and novel arrays harbouring several million SNPs will be cheap and versatile techniques for the future. It is important to consider a method that most accurately determines the SNP/CNV of relevance. In general terms there are many different protocols for the same polymorphisms. An important consideration is the number of polymorphisms to be determined for a certain gene, e.g. CYP2D6, where > 80 different alleles have been described. The genotyping will here never cover 100 % of the polymorphisms present in the population and one can estimate that analysis for the 20 most important ones will have a predictability for the phenotype of 96-98 %. Phenotyping using a probe substrate is always an alternative.

In general terms, the method should first be analytically validated utilizing well characterised standard samples carrying the polymorphism in question, preferably both in the heterozygous and homozygous states. When using PCR techniques it is important to repetitively analyse blank samples only containing water/buffer in order to exclude contamination reactions. In cases where high interindividual variability in pharmacokinetics is observed without any likely hypothesis regarding the genetic origin, it is strongly recommended to make efforts to clarify a genetic origin. Screening for those can be done using large SNP arrays or next generation sequencing efforts using isolated genomic DNA from the outlier group, in comparison to genomic DNA from controls having the normal pharmacokinetics. In cases where a significant association between a SNP/CNV and the pharmacokinetic variation in question is observed, it is important to analyse the true functional polymorphic site (see 5.3), which might be in linkage disequilibrium to the SNPs/CNVs present on the array chip or as obtained from the sequencing data. To obtain reliable data it is important to include a large enough number of samples to allow for rare alleles, in order to provide enough power for reliable statistical calculations.

Modelling and simulation methods can also help in analysing the data and designing further studies as pharmacogenetic variants can be incorporated in the models as variables of interest. Focus should be on the causative genetic alteration in question and haplotype characterisation and modelling is not required.

5.3. Genome wide association studies

Genome wide association studies (GWAS) are now commonly used for identification of the true loci of importance for interindividual differences in drug action[6,12,14,15,16]. Thus recent published results show that GWAS has been of use in the identification of variable alleles responsible for altered response or dosing toxicity of several different drugs (e.g. for simvastatin and clopidogrel). In this respect, it is advised to take note of emerging GWAS knowledge in relevant public databases[iv].

The significance of the association between the phenotype and the polymorphism must reach a high statistical level and results should, preferably, be obtained from a second independent cohort. In order

[iv] , e.g., the HuGE Navigator (http://hugenavigator.net/), the NIH Database of Genotype and Phenotype (http://www.ncbi.nlm.nih.gov/gap) or the Catalogue of Genome-Wide Association Studies (http://www.genome.gov/GWAStudies/)

to obtain such a replication cohort, the phase III trials can be designed in such a manner that the hypothesis of a defined outlier pharmacokinetic group can be validated.

The GWAS approach has drawbacks in that not an enough number of SNPs/CNVs in ADME genes are present on many older types of arrays and that association is obtained to silent or inappropriate SNPs. The validity of the GWAS technique is also dependent on the extent of phenotype difference observed. Regarding association based on GWAS studies, valuable information can be obtained by further direct sequencing of the genomic area adjacent to the polymorphism in question. Furthermore, studies aimed at defining the function of the genetic alteration identified are highly recommended. Analyses of functional properties can be done using heterologous expression systems utilizing cDNA expression plasmids or reporter plasmids for polymorphisms in the regulatory regions. When the putative functional polymorphism/CNV has been identified, the primary pharmacokinetic data has to be analysed for significance level by taking the new SNP(s)/CNV(s) into consideration. The *in vivo* importance of the new polymorphism identified can be evaluated by retrospective stratification of previously characterised data with respect to the occurrence of the polymorphisms and by prospective studies using patients selected by genotype.

6. Presentation of study results

6.1. Conventional pharmacokinetic studies

Individual data on pharmacokinetic parameters, like AUC, C_{max}, t_{max}, CL/F or CL and F, and $t_{1/2}$ in relation to genotypes should be presented. Standard descriptive statistics for each genetic subpopulation, including mean, standard deviation and range should be provided for the pharmacokinetic parameters. The parameters representing drug exposure (e.g. C_{max} and AUC) could be presented for separate subgroups (based on genotype and/or predicted phenotype) as box-whiskers-plots. The plots should include the individual data points either overlaid or next to the boxes.

The effect of genetic differences on pharmacokinetics of the investigational drug should be calculated and the relative difference in relevant pharmacokinetic parameters presented. The 90% confidence interval for the genotype effect should be presented.

If the pharmacokinetics of active metabolites has been investigated, the data should be presented in a similar way as for an active drug. If both parent and metabolite are active, the sum of the exposure of pharmacological equivalents should be presented as well.

6.2. Population pharmacokinetic analysis

Reference is made to the *Guideline on reporting the results of population pharmacokinetic analyses* (CHMP/EWP/185990/06).

6.3. Physiology-based pharmacokinetic modelling

The report of a PBPK modelling and simulation should include detailed description of the structural models, original source and justifications for both system- and drug-dependent parameters, model assumptions and their physiological and biochemical plausibility, sensitivity analyses for relevant parameters, type of error models etc. The PBPK model needs to be qualified for its purpose. In general, the performance of the model needs to be supported by relevant in vivo data. The data needed in different situations has been specified in relevant sections in this document.

6.4. Genotyping methods and Genome wide association studies

With respect to the presentation of genotyping methodologies and outcomes, reference is made to the *Note for Guidance on genomic biomarkers related to drug response: context, structure and format of qualification submissions*. ICH Topic E16 (EMAE/CHMP/ICH/380636/2009).

6.5. Phase II and III studies

If appropriate, the same applies here as described for pharmacokinetic studies. It is acknowledged that in Phase II and III studies, full pharmacokinetic data will not always be available. Still, available pharmacokinetic or population pharmacokinetic data in relation to genotypes should be listed, and standard descriptive statistics for each genetic subpopulation, including mean, standard deviation and range should be provided for the pharmacokinetic parameters.

With respect to reporting clinical data obtained with respect to pharmacogenetics, reference is made to the *Note for Guidance on genomic biomarkers related to drug response: context, structure and format of qualification submissions*. ICH Topic E16 (EMAE/CHMP/ICH/380636/2009).

7. Evaluation of the clinical consequences of genetic differences and translation into treatment recommendations

The clinical consequences of observed differences in drug exposure in genetic subpopulations depend on several factors, such as:

- the magnitude of the difference in exposure caused by the polymorphism,
- the relationship between pharmacokinetics and pharmacodynamics of the medicinal product,
- the relationship between drug exposure and clinical effect/adverse effects,
- severity of the possible adverse events and clinical consequences of loss of efficacy.

Dosing recommendations should ensure that patients receive drug treatment which is effective and safe. Unless it is reliably shown that a difference in active substance exposure has little consequences for the efficacy and safety of a drug, a genetic effect should be compensated by adjusting the dose of the drug to achieve an exposure which is shown to be effective and safe. For this purpose, either genotype- or phenotype-based dosing can be applied or individual dose titration based on Therapeutic Drug Monitoring (TDM), efficacy or adverse events. If dose titration is applied based on clinical markers, data needs to be provided supporting that satisfactory efficacy and safety is ensured in the subpopulation.

Pharmacogenetics should be considered as one of the factors affecting pharmacokinetics of a drug or active metabolite and should thus be considered integrating the effect of other intrinsic or extrinsic variables. When a polymorphism in a metabolising enzyme or transporter causes a difference in exposure which may alter efficacy or safety, the expected level of evidence for showing that the proposed treatment recommendation is suitable for the subpopulation is comparable with that required for effects of other intrinsic or extrinsic factors affecting pharmacokinetics, like weight, age, impaired renal and hepatic function or drug-drug interactions.

The evaluation of clinical consequences should be based on information available on the relationship between exposure and efficacy/safety. If possible, a well justified target range for relevant exposure parameters should be presented for the investigational drug specifying what change in exposure would justify a posology adjustment. The target range is the range of drug exposure for which satisfactory

clinical efficacy and safety has been shown. If the target range is based on active substance exposure in patients and the pharmacogenetic effect was investigated in healthy volunteers, potential differences between the pharmacokinetics of patients and healthy subjects need to be considered. The observed exposure (presented e.g., as box-whiskers plots including individual data) should be analysed with respect to target criteria taking into account the frequency of patients with lower as well as higher exposure than the target range and the clinical consequences of these deviations.

Unless the applicant convincingly shows that the exposure obtained in the genetic subpopulation with the standard dose is effective and safe, the proposed dosing recommendation in the genetic subpopulation should normalise drug exposure. Efficacy and safety in the absence of normalised drug exposure should, preferably, be based on Phase II and III data in a sufficient number of individuals exposed to the same active substance exposure. Knowledge gained from similar drugs at increased exposure is also supportive.

If the parent drug is pharmacologically active and there are *in vivo* relevant active metabolites, the exposure of these metabolites should be taken into account when proposing dose adjustments. When relevant, the active moiety can be used to develop dose adjustment (see also section 6.1). However, increased exposure of the separate substances must also be considered. The exposure of all relevant active substances should, as far as possible, be within a well tolerated range after dose adjustment.

If the proposed dose-adjustment is based on C_{min} as a surrogate for AUC, it should be taken into account that the relation between C_{min} and AUC may be altered, if the systemic metabolism of the drug is changed.

In case the genetic subpopulation is too small to allow thorough clinical investigation of proposed dose adjustment, it is recommended that the resulting individual exposure parameters obtained with the proposed treatment recommendation are estimated, as described in section 5.1, and the safety and efficacy expected of the resulting exposure evaluated.

8. Special pharmacogenetics considerations with respect to drug-drug interactions, impaired/immature organ functions and age

8.1. Drug interactions

As a general rule, genotyping of the population included in a drug-drug interaction study for a relevant gene is recommended when pharmacogenetics are expected to affect the pharmacokinetics of any of the drugs included in the study.

Polymorphisms in metabolising enzymes and drug transporters can not only affect the exposure of the pharmacologically active substances, but can also influence the size of the effect of interacting drugs (perpetrator drugs) as well as which perpetrator drugs will affect the pharmacokinetics of the active substances. If a major metabolism pathway is absent or very diminished in a subpopulation (e.g., in poor metabolisers), other metabolism pathways will be of increased importance. The consequences of inhibition of these alternative pathways on exposure should be investigated and reflected in study protocols as well as treatment recommendations, if the drug will be used in the genetic subpopulation. The change in exposure or distribution when inhibiting the alternative metabolism or transport pathway is best determined in a drug-drug interaction study including a sufficient number of carriers of the genotype investigated. However, in case a drug interaction study in the subpopulation is not practically feasible, a worst case estimation should be made based on the available *in vivo* knowledge of the quantitative contribution of separate enzymes to drug metabolism. PBPK simulations may also

be presented in parallel if the PBPK model well predicts *in vivo* data supporting the quantitative contribution of the different pathways.

8.2. Impaired or immature organ function and age

The consequences of impaired renal function may be different in genetically different subpopulations. This applies, e.g., if renal excretion is of increased relative importance in the genetic subpopulation. The exposure of active substances resulting from impaired organ function in the genetic subpopulation should be predicted through worst-case estimations and, if desirable, PBPK modelling as described above, and the clinical consequences discussed and implemented in the labelling based on the available safety data.

In some cases, the effect of age on the effect of genotype should be considered, This is particularly important if a renally cleared drug has a rather narrow therapeutic window, the main patients population is elderly and the genetic effect was determined in young healthy volunteers. The enzymes and transport proteins involved in the pharmacokinetics of a drug substance may also be quantitatively and qualitatively different in the very young paediatric patients than in adults as a consequence of developmental gene expression. Such differences are mainly expected in newborn infants, infants and toddlers (0-2 year-old children). If a significant impact of a genetic polymorphism on the pharmacokinetics of a drug substance has been established in adults, the potential consequences in the paediatric population should be considered during drug development.

9. Specific issues related to treatment recommendations based on genetically determined differences in exposure

The *Guideline on Summary of Product Characteristics (SmPC) September 2009* advices on how to present pharmacogenetic data.

Labelling text referring to genotype testing may be: *1)* for information purposes only, *2)* recommended or *3)* mandatory. This will depend on the strength of the data available and on the efficacy and safety consequences expected.

9.1. Dose recommendations

Different routes for dose adjustment can be applied:

1) Dose titration

Differences in exposure in genetic subpopulations can be managed by dose-titration in all patients based on safety and/or efficacy markers, or on TDM. If this approach is chosen, the applicant needs to show that the titration schedule is suitable for the specific subpopulation(s) as well as for the general patient population (see section 7).

2) Optional gene based dosing

When an acceptable dose can be reached without genotyping for the relevant gene, but genetics might aid in individual dose optimisation, an approach such as safety-based titration can be enriched with an optional or advisable genetic component (e.g. with algorithms for thiopurine S-methyltransferase (TPMT) variants and 6-mercaptopurine dosing in acute lymphatic leukaemia).

3) Dosing based on genotype

If a dose titration is not satisfactory or feasible and the exposure obtained in the genetic subpopulation has not been shown to be effective and safe, the genotype should be determined by a validated

method before initiation of therapy and appropriate dose adjustments should be recommended for each relevant genetic subpopulation. If it is not possible to administer appropriate doses with the available formulation strengths, a contraindication should be considered based on the benefit-risk ratio of the treatment for the population concerned. The applicant is then encouraged to develop suitable formulations to allow dose adjustment.

In both cases 2 and 3, efforts should be made to provide clear information and recommendations to the prescriber. When relevant, recommendations should be provided in Section 4.2. In most situations it is sufficient to indicate the phenotypes (e.g. poor, extensive, ultrarapid metabolisers) in section 4.2, with reference to section 5.2. In section 5.2, detailed information on the effect of different genotypes on active substance exposure should be included and, if relevant, the effect on pharmacodynamics in section 5.1 of the SPC.

9.2. Other labelling consequences

If a suitable dose can not be recommended based on available data, this should be reflected in the SPC, e.g. as warnings, contra-indications, etc.

The frequencies of the alleles of interest in ethnic populations should be presented in the SPC section 5.2.

If genotyping is recommended, or optional, this should also be mentioned in the Product Information Leaflet (PIL).

Glossary

active metabolites	metabolites that are involved in efficacy
ADME	absorption, distribution, metabolism and excretion
allele	a variant of the DNA sequence at a given locus one of a particular gene
AUC	area under the plasma concentration-time curve
CL	clearance
C_{max}	peak concentration
CNV	copy number variation
DNA	deoxyribonucleic acid
F	absolute bioavailability
functionally polymorphism	a polymorphism that has been shown to alter enzyme or protein activity and/or the clinical disposition of drugs
gene	a locatable region of genomic sequence, corresponding to a unit of inheritance
genetic subpopulation	subdivision of the whole population, with common, distinguishing genetic characteristics. These characteristics may include both the phenotype, e.g. poor metaboliser, as well as the genotype, e.g., CYP2D6*4
GWAS	genome wide association study
haplotype	a combination of alleles at different loci on the chromosome that are transmitted together
locus	the specific location of a gene or DNA sequence on a chromosome
normalised exposure	an exposure in a genetically defined subgroup which is comparable to the exposure in the main population. obtained by an adjusted dose
PBPK	physiologically based pharmacokinetics
PD	pharmacodynamics
perpetrator drug	drug that affects metabolism or transport of the other drug
pharmacogenetics	the study of variations in DNA sequence as related to drug response
PIL	product information leaflet
PK	pharmacokinetics
RNA	ribonucleic acid
RT-PCR	real time polymerase chain reaction
SNP	single nucleotide polymorphism
SPC	summary of product characteristics
$t_{1/2}$	elimination half-life

TDM	therapeutic drug monitoring
t_{max}	time when C_{max} occurs
toxic metabolite	metabolite that is related to adverse events, i.e., related to safety, often due to off-target effects

References

[1] Pharmacogenomics: translating functional genomics into rational therapeutics. Evans WE, Relling MV. Science. 1999; 286:487-491

[2] Pharmacogenomics and individualized drug therapy. Eichelbaum M, Ingelman-Sundberg M, Evans WE. Annu Rev Med. 2006; 57:119-137.

[3] Pharmacogenetics and human genetic polymorphisms. Daly AK. Biochem J. 2010; 429:435-449.

[4] Pharmacogenomic biomarker information in drug labels approved by the United States food and drug administration: prevalence of related drug use. Frueh FW, Amur S, Mummaneni P, Epstein RS, Aubert RE, DeLuca TM, Verbrugge RR, Burckart GJ, Lesko LJ. Pharmacotherapy. 2008; 28:992-998.

[5] Pharmacogenomic biomarkers: new tools in current and future drug therapy. Sim SC, Ingelman-Sundberg M. Trends Pharmacol Sci. 2011; 32:72-81.

[6] Integration of genetic, clinical, and INR data to refine warfarin dosing. Lenzini P, Wadelius M, Kimmel S, Anderson JL, Jorgensen AL, Pirmohamed M, Caldwell MD, Limdi N, Burmester JK, Dowd MB, Angchaisuksiri P, Bass AR, Chen J, Eriksson N, Rane A, Lindh JD, Carlquist JF, Horne BD, Grice G, Milligan PE, Eby C, Shin J, Kim H, Kurnik D, Stein CM, McMillin G, Pendleton RC, Berg RL, Deloukas P, Gage BF. Clin Pharmacol Ther. 2010; 87:572-578.

[7] A genome-wide association study of acenocoumarol maintenance dosage. Teichert M, Eijgelsheim M, Rivadeneira F, Uitterlinden AG, van Schaik RH, Hofman A, De Smet PA, van Gelder T, Visser LE, Stricker BH. Hum Mol Genet. 2009; 18:3758-3768.

[8] Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. Mega JL, Simon T, Collet JP, Anderson JL, Antman EM, Bliden K, Cannon CP, Danchin N, Giusti B, Gurbel P, Horne BD, Hulot JS, Kastrati A, Montalescot G, Neumann FJ, Shen L, Sibbing D, Steg PG, Trenk D, Wiviott SD, Sabatine MS. JAMA. 2010; 304:1821-1830.

[9] Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, Damcott CM, Pakyz R, Tantry US, Gibson Q, Pollin TI, Post W, Parsa A, Mitchell BD, Faraday N, Herzog W, Gurbel PA. JAMA. 2009; 302:849-857.

[10] SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. Pasanen MK, Neuvonen M, Neuvonen PJ, Niemi M. Pharmacogenet Genomics. 2006; 16:873-879

[11] Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics. Ho RH, Tirona RG, Leake BF, Glaeser H, Lee W, Lemke CJ, Wang Y, Kim RB. Gastroenterology. 2006; 130:1793-1806

[12] SLCO1B1 Variants and Statin-Induced Myopathy — A Genomewide Study. The SEARCH Collaborative Group. N Engl J Med 2008; 359:789-799

[13] Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. Stearns V, Johnson MD, Rae JM, Morocho A, Novielli A, Bhargava P, Hayes DF, Desta Z, Flockhart DA. J Natl Cancer Inst. 2003; 95:1758-1764

[14] Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. Treviño LR, Shimasaki N, Yang W, Panetta JC, Cheng C, Pei D, Chan D, Sparreboom A, Giacomini KM, Pui CH, Evans WE, Relling MV. J Clin Oncol. 2009; 27:5972-5978.

[15] HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, Daly MJ, Goldstein DB, John S, Nelson MR, Graham J, Park BK, Dillon JF, Bernal W, Cordell HJ, Pirmohamed M, Aithal GP, Day CP; DILIGEN Study; International SAE Consortium. Nat Genet. 2009; 41:816-819.

[16] IL28B in hepatitis C virus infection: translating pharmacogenomics into clinical practice. Ahlenstiel G, Booth DR, George J. J Gastroenterol. 2010; 45:903-910.