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

Draft

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

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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. In particular, guidance is given regarding studies required and recommended at different phases of drug development to ensure satisfactory efficacy and safety in pharmacogenetic subpopulations that have variable systemic exposure of active substances.

1. Introduction (background)

The pharmacokinetics of many medicinal products is prone to interindividual variability, which is caused by several factors such as gender, age, weight, 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, where 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. A great deal of this interindividual variability is caused by genetic polymorphism, i.e. the occurrence in the same population of multiple allelic states. Genetic variations are demonstrated by the identification of Single Nucleotide Polymorphisms (SNPs), insertions/deletions and variation in gene copy number (copy number variation, CNV).

With respect to pharmacokinetics, the highest level of polymorphism is found in genes involved in drug metabolism; phase I metabolism of approximately 40% of clinically used drugs is due to polymorphic enzymes. Currently, the most important polymorphic enzymes are the cytochrome P450 enzymes such as CYP2C9, CYP2C19 and CYP2D6. Subjects who have extensive and poor metabolising capacity for these enzymes are present in the general population. For CYP2D6, besides the poor metaboliser phenotype, the ultrarapid metaboliser phenotype is relevant as well. With respect to phase II enzymes, the genetic variability of UDP-glucuronosyltransferases, *N*-acetyltransferase-2 and some methyltransferases has been linked to interindividual pharmacokinetic variability. The metabolising enzymes account for 80% of the drugs which currently include pharmacogenetic data in their labelling.

Among the major clinically relevant issues is pharmacogenetic variability causing increased or decreased metabolism of the parent drug and the subsequent formation of active or toxic substances. Decreased metabolism can cause too high levels of the parent drug and adverse drug reactions. Elevated drug metabolism can cause loss of response or, in case of prodrug activation, too high levels of the bioactive compound.

The interindividual genetically linked differences in pharmacokinetics may cause, clinically, very relevant alterations in drug action. Optimal efficacy is dependent on appropriate dosing, often based on the specific genotype. Thus, the effective dose may vary greatly due to increased or decreased drug elimination rate. For instance, due to CYP2D6 polymorphism, the rate of hepatic metabolism of drugs which are substrates for this enzyme can vary 1000-fold between individuals. Among many antidepressants and antipsychotics, the plasma levels of the drug at the same dosage often vary 5-20-fold. A 9-fold higher risk of suicide has been reported among ultrarapid metabolisers of CYP2D6 and there are also many reports of increased frequency of adverse drug reactions among subjects with the poor metaboliser phenotype, due to increased systemic exposure of the parent drug. Furthermore, increased side effects after treatment with analgesic drugs, which are activated by CYP2D6, are seen among ultrarapid metabolisers. The efficacy of prodrugs which are activated by polymorphic enzymes varies depending on the pharmacogenetics of the patients. An example of this is clopidogrel, for which an increased frequency in serious side effects due to excessive prodrug activation has been seen in patients with increased formation of the active metabolite and a corresponding lack of effect in subjects without the appropriate enzyme. Dosing of some important anticoagulants is dependent on the CYP2C9 genotype of the patient and the platelet inhibition action of some drugs is dependent on the CYP2C19 genotype. Overall, it can be estimated that 20-25 % of the efficacy of all drug treatment is significantly affected by interindividual differences in genes encoding drug metabolising enzymes.

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. The effect of transporter polymorphism on drug pharmacokinetics is thought to be of less importance, or is still unknown, compared with polymorphic enzymes. This is partly due to the fact that the role of specific transporters *in vivo* is difficult to quantify due to the lack of specific inhibitors

and the polymorphism seen in the transporters is often substrate specific in its effects. The possibility of transporter polymorphism as a cause of altered pharmacokinetics must, however, always be considered in all phases of drug development. It is anticipated that this area will expand a great deal in the near future, as knowledge of the role of drug transporters is rapidly developing.

At present, an increasing proportion of lead compounds selected for further development are metabolised by enzymes or transported by transporter proteins upon which the impact of pharmacogenetics is unknown. New technologies, such as rapid genome sequencing, whole genome wide association studies (GWAS) and targeted absorption, distribution, metabolism and excretion (ADME) gene SNP/CNV analyses, are expected to have an integral role in clinical drug development in the future.

Until now, it has been difficult to transfer knowledge of the effect of polymorphism into specific recommendations in affected genetic subpopulations[1]. In this respect, genetic subpopulations have been treated differently than other subpopulations or circumstances in which the exposure of active or toxic substances is increased. 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. This document, therefore, aims to clarify the studies needed to investigate these issues.

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.
The following issues are discussed in this guideline:

- In which situations and at what stage(s) in the clinical development program should pharmacogenetic/pharmacokinetic studies be performed.
- Recommendations regarding pharmacogenetic/pharmacokinetic studies investigating the effects of polymorphisms at ADME level (enzymes and transporters), 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 genetic differences on pharmacokinetic parameters for treatment recommendations and labelling.
- Special considerations related to drug-drug interactions and the effect of impaired or immature organ functions on pharmacogenetics.

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, Module 5 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:

- Pharmacokinetic studies in man (Notice to applicants, Vol 3C, C3a, 1987).
- Guideline on reporting the results of population pharmacokinetic analyses (CHMP/EWP/185990/06).
- The investigation of drug interactions (CPMP/EWP/560/95).
- Guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98 Rev.1).
- Guideline on the role of pharmacokinetics in the development of medicinal products in the paediatric population (EMEA/CHMP/EWP/147013/2004).

[1] The term “genetic subpopulation” may include both the qualifiers of the polymorphisms, e.g. poor metaboliser, as well as the indication of the concerned allele, e.g., CYP 2D6*4.

- 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.
- ICH Topic E15. Note for Guidance on definitions for Genomic biomarkers, pharmacogenomics, pharmacogenetics, genomic data and sample coding categories (EMEA/CHMP/ICH/437986/2006).
- ICH Topic E16. Note for Guidance on genomic biomarkers related to drug response: context, structure and format of qualification submissions.(EMEA/CHMP/ICH/380636/2009).

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

4.1. General recommendations

Pharmacogenetic variants often influence drug pharmacodynamics but also the absorption, distribution, metabolism and excretion of a drug. Furthermore, pharmacogenetics may also influence the formation and elimination of metabolites and this should be remembered if there are metabolites that may affect the efficacy and/or safety of the drug. Genotypes leading to absent, decreased or increased enzyme or transport protein activity 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 are generally **required** during development:

a) If *in vitro* studies and/or *in vivo* studies indicate an important role for a functionally polymorphic enzyme in the metabolism of the drug, *or*

b) If *in vitro* studies and/or *in vivo* studies indicate an important role for a functionally polymorphic enzyme in the formation or elimination of a pharmacologically active or toxic metabolite, or

c) If *in vivo* studies indicate that a transport protein known to be subject to clinically relevant polymorphism has a significant effect on the systemic or target organ exposure of pharmacologically active drug or metabolites.

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:

a) if available *in vitro* data indicate that a polymorphic enzyme or transporter contributes to the pharmacokinetics of the active substances but the quantitative role is unknown, *or*

b) if there is high interindividual pharmacokinetic variability, or there are pharmacokinetic outliers with several-fold higher or markedly lower exposure to the active substances, which gives rise to clinical efficacy and safety concerns based on the existing knowledge, *or*

c) if safety concerns are observed which may be related to genetic differences in systemic or target organ exposure, *or*

d) if major differences in pharmacokinetics are observed in different ethnic groups.

If a studied polymorphism of an enzyme or transporter does not affect the functional performance of the protein, genotyping[2] 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.

Still, in all clinical phases of development, prospective sampling of DNA for genotype analyses is 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

[2] In this guideline the term genotyping is used. However, phenotyping by, for example, catalytic assays may also be an acceptable approach

importance can be identified later and that unknown but important metabolic pathways in which a polymorphism might have an effect, may be identified at later stages in the programme.

4.2. Integrating pharmacogenetic effects on pharmacokinetics in drug development

Below, recommendations are made on how to implement pharmacogenetics during the different phases of clinical development, starting with the *in vitro* studies conducted before studying the medicinal product in man.

4.2.1. In vitro studies prior to human exposure.

Prior to Phase I, human *in vitro* metabolism studies should be performed. Such studies should preferably include identification of the enzymes catalysing the *in vitro* metabolism and also the identification and characterisation of metabolites formed, enabling pharmacological activity screening of these metabolites. It should be remembered that polymorphic enzymes can participate in the formation and elimination of pharmacologically active metabolites of the drug, including toxic metabolites. Even when these pathways quantitatively represent a minor pathway for drug elimination, they may be highly significant in terms of safety or efficacy. Based on these data, the involvement of known functionally polymorphic enzymes in the elimination of the parent compound and/or the formation and elimination of active metabolites should be determined. As *in vitro* studies are not always quantitatively predictive of the *in vivo* situation, the enzyme involvement needs to be confirmed *in vivo*.

The involvement of transporters may be indicated by *in vitro* data obtained prior to the start of Phase I. However, it is presently very difficult to make quantitative predictions of the *in vivo* contribution of transporters. The *in vivo* importance of a transporter may be demonstrated by preclinical *in vivo* information or information on similar substances.

4.2.2. First time in man studies

The possibility of genetic influence should be considered early in the Phase I program. When the *in vitro* data indicate that involvement of a known functionally polymorphic enzyme cannot be excluded, it is advised to genotype the first time in man study population. If the available *in vitro* data strongly indicate major involvement of a polymorphic enzyme in the metabolism and/or formation of pharmacologically active or toxic substances, subjects with a genotype predicted to result in markedly increased exposure of active or toxic 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 proteins is not mature enough to estimate the potential for significant involvement of the transporter *in vivo* based on *in vitro* data. Unless there are other indications of significant transporter involvement *in vivo*, early genotyping is not required on the basis of *in vitro* data. Still, prospective storing of samples in order to allow eventual pharmacogenetic analysis is 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.

4.2.3. Phase I

When based on available *in vitro* or preliminary clinical data, genotype is predicted or known to markedly affect the pharmacokinetics of pharmacologically active compounds, genotyping is recommended 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 genetic subpopulation(s).

In Phase I, the relative contribution of the identified polymorphic enzyme to total body clearance should be estimated. If feasible, it is recommended to investigate this in a conventional pharmacokinetic study with targeted inclusion of subjects of certain genotypes (see section 5.1). If this is not feasible, but potent and sufficiently specific *in vivo* inhibitors of the enzyme are available, the effect of the polymorphism should be estimated using the results of an interaction study with such an

inhibitor. The effect of the identified polymorphism on the maximum tolerated dose should also be determined on the basis of these studies.

If a marked effect of polymorphism is confirmed, it is recommended, if feasible, to expand the clinical Phase 1 program and also evaluate the interaction potential, as well as the consequences of impaired organ function and age in the genetic subpopulations (see sections 8.1 and 8.2). Furthermore, dose-linearity in poor metabolisers at relevant doses should be investigated. If pharmacokinetics are linear in the general population, these data may be extrapolated to populations with increased protein activity. However, if pharmacokinetics are nonlinear in extensive metabolisers, it is recommended to investigate dose-proportionality in ultrarapid metabolisers, in order to provide satisfactory background knowledge for the future studies. This evaluation should preferably be done before starting Phase II and the results of this evaluation should be taken into consideration in the study protocol.

4.2.4. Phase II (dose finding, exploratory)

If Phase I studies strongly indicate that pharmacogenetics may influence the pharmacokinetics of a drug to a clinically relevant extent, this should be considered in the design of the Phase II studies. It is then required to genotype the included patients. Genotype based dosing or exclusion of certain genotypes should be considered. The aim should be to minimise variability in exposure of pharmacologically active substances for each dose-level studied and to optimise target dose selection in the Phase III studies. The effect of the known polymorphism on pharmacokinetics, safety and efficacy in the populations of altered genotype should be clarified. Dose recommendations for genetic subpopulations should be worked out, based on, for instance, the pharmacokinetic characteristics and pharmacodynamics of the new active substance. The expected level of evidence for a genotype-based dose adjustment is comparable with that required for dose adjustment based on other intrinsic and extrinsic factors affecting pharmacokinetics, like weight, age, renal and hepatic impairment or drug-drug interactions. If active substance exposure in Phase III is not “normalised” through genotype based dosing, dose titration or, less desirably, through genotype based exclusion of patients, sufficient data needs to be collected on the consequences of the altered exposure on the clinical efficacy and safety of the drug (see section 7).

4.2.5. Phase III (confirmatory)

If available data indicate that there is a significant difference in drug exposure in the pharmacogenetic subpopulation, all patients included in phase III studies should be genotyped. Depending on the likely consequences of the polymorphism for efficacy or safety, there are several ways that this knowledge could be influence the design of Phase III studies:

a) No genotype-specific treatment (if the available data indicate that the difference in exposure may not be clinically relevant, so there are no major safety or efficacy concerns related to the difference in exposure).

b) Normalising the exposure of active substances through genotype based dosing.

c) Dose titration regardless of genotype (in case a suitable marker exists).

d) Exclusion of patients of a specific genotype (i.e., exclude patients at risk, if it is not possible to normalise the exposure).

In case the exposure of active substance(s) is not normalised through genotype-based dosing, the conclusion of similar efficacy and safety at the exposure obtained in the subpopulation needs to be supported by sufficient clinical data obtained at these exposure levels to allow these conclusions. This may included both Phase II and Phase III data.

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 subpopulations.

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

In Section 4.2 of this guideline the ideal situation has been described, where the potential effect of pharmacogenetics is detected early in drug development. In situations where there are no predictive indications of pharmacogenetic effects on the pharmacokinetics of the drug when initiating the clinical part of the development program of a new medicinal product, the acquired pharmacokinetic, clinical

efficacy and safety information at a certain stage in the drug development may indicate the need for investigations of the pharmacogenetic impact on drug or metabolite exposure. This situation may occur:

a) when a previously unknown or sparsely studied 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 is known but there was no prior knowledge regarding polymorphisms of the gene,

c*)* if knowledge of the importance of an enzyme or transporter to the pharmacokinetics of a drug is gained at a late stage in the development program,

d*)* if high interindividual pharmacokinetic variability, or marked outliers in drug exposure are observed,

e) if there are safety or efficacy concerns observed which may originate from pharmacogenetic differences in local tissue or systemic drug exposure, or,

f) if there are major differences in the pharmacokinetics of the medicinal product between different ethnic groups.

See section 4.1 for situations in which pharmacogenetic investigations are required or recommended.

If data become available, during the clinical development program or during pharmacovigilance monitoring, demonstrating the involvement of a polymorphic enzyme or transporter as a cause for important inter-individual variations in pharmacokinetics, it is recommended to, as far as possible, estimate the relative contribution of the metabolism or transport pathway in question to the bioavailability and/or clearance of the medicinal product *in vivo*. If needed, it is recommended that relevant *in vitro* studies are carried out in which the direct influence of the polymorphic variant enzyme is characterised. See also section 4.2.

If the genetic locus associated with the identified pharmacokinetic variability influencing clinical or safety aspects is unknown, it is advisable to carry out analysis (such as phenotype-genotype associations) of genes potentially responsible for the variation. More thorough investigations using modern techniques such as GWAS, genome sequencing, or array based SNP/CNV investigations are recommended to identify the background (see sections 5.2 and 5.3).

Conclusions from a retrospective analysis carried out in response to emerging data may be acceptable if mechanistically supported by available pharmacokinetic information. In this case, DNA should preferably be available from a large proportion of patients. 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 pharmacokinetic consequences of this finding, are recommended.

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 requirements during drug development with regard to monitoring pharmacogenetics related to pharmacokinetics depend on the stage of the clinical development plan and how strong the evidence is for a clinically relevant effect of pharmacogenetics on drug exposure. *In silico* Physiologically Based Pharmacokinetic (PBPK) modelling and simulation may be helpful when optimising the design of *in vivo* pharmacokinetic studies.

The effect of polymorphism on the exposure of pharmacologically active substances should, if possible, be investigated in a pharmacokinetic study of conventional, frequent blood sampling, design. A Phase I study of reduced design, including the extremes of genotypes (e.g., extensive metabolisers vs. poor and/or ultra-rapid metabolisers) is usually performed as a basis for the evaluation of the pharmacogenetic effect on active substance exposure. However, if a likely clinically relevant effect is found, the consequences of having an intermediate protein activity should also be investigated. The study may be of single-dose design. If the pharmacokinetics are nonlinear and a dose range is recommended, the highest and lowest dose of the range should be covered (see section 4.2.3). If an effect of genotype is observed in the single-dose study, a multiple dose study is needed unless dose

linearity has been shown at the concentrations obtained in carriers of the low activity (e.g. poor metabolisers) genotypes and the pharmacokinetics of the drug is independent of time.

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 reduced or highest activity are difficult to recruit as the allele is very rare, as many carriers of the rare extreme genotype as possible should be included. In this case, the study may include 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. In this respect, available data from other drugs may be helpful to support the relation between genotype and phenotype.

Population pharmacokinetic analysis of datasets, with or without the inclusion of rich data sets, could be used to confirm the effect of pharmacogenetics on active substance exposure. It may also be used as a hypothesis-generating tool where the effect of new or unexpected polymorphisms may be indicated. The study population should include a satisfactory number of patients of each genotype, and enough samples per patient to obtain valid estimates. Power calculations and sampling optimisation should be done before the initiation of the study in order to ensure a sufficient population size. If the data and analysis is of high quality and the study sufficiently powered, the data may be used to support labelling.

However, if a need for pharmacogenetics based dose adjustments has been identified, this should, generally, be supported by a conventional pharmacokinetic study, as in such cases a more 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.

5.2. Genotyping methods and choice of alleles

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 (PCR), oligonucleotide ligation assay (OLA), TaqMan PCR, SNP/CNV arrays, pyrosequencing, genomic sequencing, etc. In the case of targeted genotyping, the method should first be validated utilizing well characterised standard samples carrying the mutation in question, preferably both in the heterozygous and homozygous states. When using PCR techniques it is important to continuously analyse blank samples only containing water/buffer in order to exclude contamination reactions. The polymorphisms studied should, in principle, be those where the genetic alteration causes changes in gene function or be strongly associated with documented phenotypic variants. 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. The genetic analyses should, preferably, also encompass assays for CNVs, a type of polymorphism of increasing importance.

In cases where high interindividual variability in pharmacokinetics is observed without any likely hypothesis regarding the genetic origin, commercial arrays for analyses of the 10,000- 30,000 most relevant SNPs and CNVs in ADME genes are now available and could provide relevant results regarding the genetic origin of the pharmacokinetic variation. In cases where a significant association is made to the pharmacokinetic variation in question, it is important to analyse the true functional polymorphic site, which might be in linkage disequilibrium to the SNPs/CNVs present on the chip. Modelling and simulation methods can help in analysing the data and designing further studies as pharmacogenetic variants can be incorporated in the models as variables of interest.

5.3. Genome wide association studies

In cases where the relevant hypotheses for the genetic basis of interindividual variation in pharmacokinetics cannot be placed, genome wide association studies (GWAS) now offer an alternative method for identification of the true locus of importance. Today SNP/CNV arrays monitoring more than 1.1 million genetic alternations in the human genome are commercially available. Such arrays are now more routinely used for identification of disease causing genes and have also been useful in the identification of genetic variations e.g. in the MHC Class II region responsible for increased sensitivity to adverse drug reactions caused by certain drugs. Note should be taken of emerging GWAS

knowledge in relevant public databases[3]. In case no explanation for observed variability is found, GWAS studies to reveal interindividual differences in pharmacokinetics and pharmacodynamics of drugs are recommended in the clinical development of drugs. 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. The significance of the linkage between the phenotype and the mutation/CNV must reach a high statistical level and results should, preferably, be obtained from a second independent cohort. Furthermore, the SNP associated with the phenotype may not be the true causative mutation. In these cases it is recommended to sequence the loci in the neighbouring regions, analyse the SNPs linked to the initial SNP detected and analyse the data for significance level by taking the new SNP(s) into consideration. If the significance level is increased, the functional aspects of the mutation have to be analysed. The new mutations discovered in these studies should, preferably, be validated *in vitro* and *in vivo*. The functional consequences can be identified using cellular transfection of variant plasmids. The *in vivo* importance can be evaluated by retrospective stratification of previously characterised data with respect to the occurrence of the mutations 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 listed. 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 as box-whiskers-plots. The plots should include the individual data points either overlaid or next to the boxes.

The effect of pharmacogenetics 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 for the metabolites. If suitable, active moiety, i.e. the sum of the unbound exposure of pharmacological equivalents, should be presented in addition to the effects of the separate substances. However, this estimation is only correct if the distributions of parent drug and metabolite to the target site(s) are similar. The validity of this assumption should be discussed and, if possible, the calculations could be modified by the metabolite to parent target organ distribution ratio.

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. 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).

6.4. 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.

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

With respect to 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 effects/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. Preferably, 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. Either genotype-base dosing is applied or the dose individualisation is performed through individual dose titration based on Therapeutic Drug Monitoring (TDM), efficacy or adverse events.

When a polymorphism in a metabolising enzyme or transporter leads to 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. 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 drug 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 (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 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.

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 and the safety and efficacy expected of the resulting exposure is evaluated as described in section 5.1.

In general, clinical impact and treatment recommendations should be evaluated separately for the homozygous and heterozygous population, since in most cases clinical consequences are expected to differ between these subgroups.

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 elimination of the drug is changed.

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

8.1. Drug interactions

Polymorphisms in metabolising enzymes can not only affect the exposure of the pharmacologically active substances, but also impact on the drug-drug interaction profile. Therefore, the possible effects of polymorphisms on the interaction profile should be evaluated.

If a major elimination pathway is absent or very diminished in a subpopulation (e.g., in poor metabolisers), other elimination pathways will be of increased importance. The consequences of inhibition of these alternative pathways should be determined and reflected in study protocols as well as treatment recommendations, if the drug will be used in the subpopulation. In case a drug interaction study in the subpopulation is not possible, a worst case estimation should be made based on the available *in vivo* knowledge of the quantitative contribution of separate enzymes to drug elimination. If the subpopulation is not identified (e.g., through genotyping) before combining the interacting drugs, the general treatment recommendations should be suitable for the subpopulation. The potential need to adjust any treatment recommendation related to the effects of other drugs on the pharmacokinetics of the drug under investigation, or vice versa, should be considered.

As a general rule, genotyping of the population included in a drug-drug interaction study is recommended when pharmacogenetics are expected to affect the pharmacokinetics of any of the drugs included in the study. Depending on the aim of the study, a list of inclusion or exclusion criteria may contain specific genotypes.

8.2. Impaired or immature organ function and age

The consequences of impaired organ function may be different in genetically different subpopulations. This applies if the main elimination pathway in the genetic subpopulation is markedly affected by the impaired organ function. The exposure of active substances resulting from impaired organ function in the genetic subpopulation should be estimated and the clinical consequences discussed based on the available safety data. Additional information should be included in the SPC to reflect this.

The enzymes and transport proteins involved in the pharmacokinetics of a drug substance may 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 further elaborated and taken into account during drug development. Likewise, it should be considered that in the very elderly patients, pharmacogenetic related differences in metabolism could occur and have a different impact on efficacy and safety, due to impaired functionality of system/organs.

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

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

If the expected consequences of having a different exposure of active substances due to genetic polymorphism are undesirable, for instance, adverse events affecting quality of life, but not sufficiently severe for mandatory genotyping to be required, the option of improving the benefit/risk ratio via genotyping prior to treatment should be mentioned in the SPC. In this case, suitable treatment recommendations should be given for the genetic subpopulation.

When knowledge is available that genetics might aid in individual dose optimisation, an approach such as safety-based titration can be enriched with a 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 safety or efficacy consequences of the exposure difference in the subpopulation are considered to be a major concern, the genotype should be carefully ascertained 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 case 2 and 3, effort should be made to provide clear information and recommendations to the prescriber. When relevant, recommendations shall 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. In case 2, available information on the difference in adverse events should be presented in section 4.8.

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.

Well-documented functional polymorphisms that have not been studied because of their rare appearance should be reflected in the SPC if they are likely to influence drug exposure to a clinically relevant extent.

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).