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Guideline on good pharmacogenomic practice

Draft

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Guideline on good pharmacogenomic practice

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Executive summary

Genomic data have become important in the evaluation of efficacy and safety of drugs for regulatory approval, and in guiding patient treatment in the clinic resulting in inclusion of information on genomic biomarkers in drug labels where relevant. The integration of genomic biomarkers in clinical trials and other studies, as well as the technology used, should follow certain principles in order to generate reliable evidence for decision making and patient treatment.

The influence of the biomarkers on the studies, their analyses and outcome should be considered, with the intention to maximise the benefit and/or minimise risks for patient treatment.

Although the International Council for Harmonisation (ICH) E15 and E16 and European Medicines Agency (EMA) guidance describe some principles for the regulatory evaluation of genomic biomarkers, there is currently no guideline on good genomic practices. The intention of this guidance is to increase the usefulness of the information gathered from genomic studies and facilitate the implementation of pharmacogenomics (PGx) into drug development and patient treatment for the benefit of all stakeholders.

The aim of this guideline is to lay out the requirements related to the choice of appropriate genomic methodologies during the development and life-cycle of a drug. Problems encountered in previous studies evaluating genetic variation in drug response are reviewed including the complex relationship between determination of phenotype vs the identified genotype with respect to defining drug response and use in drug development. With the continuing developments in genomic technologies, we also include sections on (a) emerging knowledge of epigenetic alterations and the future usefulness of epigenetic alterations in tumour DNA as predictors for drug resistance and response; (b) increasing awareness of the importance of rare mutations in drug response together with a comparison of the different methods for DNA sequencing; (c) the importance of sample preparation and methods for evaluation of mutations and Copy Number Variations (CNV) of relevance for pharmacotherapy; (d) the design of in vivo studies including randomized controlled trials (RCT) for analyses of the influence of genetic variation for adverse drug reactions and response; and (e) the translation of knowledge of pharmacogenomic biomarkers into the clinics including the relevance of pharmacogenomic drug labels.

1. Scope

The scope of this guideline comprises requirements related to the choice of appropriate genomic methodologies during the development and the life-cycle of a drug. Principles for a robust clinical genomic dataset are discussed and key scientific and technological aspects for the determination and interpretation of the genomic biomarker data and their translation into clinical practice are highlighted.

Novel biomarkers, including RNA, circulating DNA, miRNAs or proteins/peptides that can predict drug response, are becoming increasingly important for personalized medicine. However, the focus of this guideline are biomarkers originating from genomic DNA, and we therefore do not cover other types of biomarkers

2. Legal basis and relevant guidelines

This guideline applies to Marketing Authorization Applications for medicines for human use and should be read in conjunction with all other relevant EU and ICH guidelines as well as reflection papers. These include, but are not limited to:

- Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products - EMA/CHMP/37646/2009
  http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/02/WC500121954.pdf
- Guideline on key aspects for the use of pharmacogenomics in the pharmacovigilance of medicinal products - EMA/CHMP/281371/2013
  http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/11/WC500196800.pdf
- Reflection paper on methodological issues associated with pharmacogenomic biomarkers in relation to clinical development and patient selection - EMA/446337/2011
  http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/07/WC500108672.pdf
- Reflection paper on co-development of pharmacogenomic biomarkers and Assays in the context of drug development- EMA/CHMP/641298/2008
  http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/07/WC500094445.pdf
- Guideline on the evaluation of anticancer medicinal products in man - EMA/CHMP/205/95/Rev.4
  http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/01/WC500137128.pdf
- Reflection paper on pharmacogenomics in oncology - EMEA/CHMP/PGxWP/128435/2006
  http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003866.pdf
- Reflection paper on pharmacogenomic samples, testing and data handling - EMEA/CHMP/PGxWP/201914/2006
  http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003864.pdf
- Note for guidance on definitions for genomic biomarkers, pharmacogenomics, pharmacogenetics, genomic data and sample coding categories - EMEA/CHMP/ICH/437986/2006 (ICH Topic E15)
  http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002880.pdf
- Position paper on terminology in pharmacogenetics - EMEA/CPMP/3070/01
  http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003889.pdf
- The Rules Governing Medicinal Products in the European Union Volume 2C Notice to Applicants; A guideline on summary of product characteristics (SmPC) September 2009
  http://ec.europa.eu/health/files/eudralex/vol-2/c/smpc_guideline_rev2_en.pdf

3. Background

The concept of "Personalised Medicine" has received much attention in recent years. There has been an increase in our understanding of inter-individual differences in DNA sequences, as well as the ability to link drug response to variation in the human genome. As a consequence, pharmaceutical industry, academia, clinicians and regulators are now focusing more on the genomic basis for individual response. This leads to a transition from population-based prescribing to more individualized treatment both in clinical drug development and practice. Pharmacogenomics include genomic and epigenomic factors influencing drug pharmacokinetics (PK), pharmacodynamics (PD), drug efficacy and safety as well as drug-drug-interactions (DDIs).

The identification of genomic factors influencing variability in drug response has focused mainly on variation in genes encoding: (i) drug-metabolising enzymes (cytochrome P450 enzymes, (CYPs), phase II enzymes), (ii) drug transporters, and (iii) drug targets (e.g. receptors, signal transduction molecules). This primarily includes analyses of the germline (host) genome but also of the somatic genome of tumours, or of the genome of infectious agents. For prediction of adverse drug reactions, in certain situations, analyses of specific (host) human leukocyte antigen (HLA) haplotypes is also of importance.

Research in the area of pharmacogenomics has revealed many important variable genetic loci that influence drug response. However, in addition, a proportion of clinical studies conducted have resulted in ambiguous findings highlighting the importance of correct measurement, determination, interpretation and translation of pharmacogenomic data into clinical treatment. Important pitfalls identified in published studies include:

- Poor quality of the employed analytics
    - Analyses of non-relevant Single Nucleotide Variations (SNVs)
    - Analysing somatic instead of germline DNA when germline DNA analysis is intended
- Lack of appropriate patient selection
- Lack of appropriate phenotype identification
- Lack of power in relation to the frequency of the genetic variation studied
- Using non-PGx design for making claims on PGx markers
- Non relevant endpoints selected for the basis of the study
- Failure to take into account the pharmacology of the drug in the design of the study

Genomic studies, irrespective of whether they are conducted by academia or industry and/or for research and/or regulatory purposes, should be conducted using good genomic practices which will enable data comparison, integration and most efficient use.

Examples highlighting the need for harmonised good genomic practices include:

- Studies regarding the importance of *CYP2C19* polymorphism for the efficacy and adverse effects of clopidogrel in relation to comparators have had different kinds of patient populations in different studies, leading to discrepant results.
- Similarly, large prospective randomized studies of the impact of CYP2C9 and VKORC1 on the treatment with vitamin K antagonists have had different designs including different algorithms used for dosing, differences in the ethnicity and frequencies of the relevant mutations among patients recruited in the different trials, also causing different outcomes.
- In another study, two drugs, acenocoumarol and phenprocoumon, were combined together in one group, despite the fact that mainly acenocoumarol has been shown to be influenced by the polymorphism in the CYP2C9 and VKORC1 genes. Furthermore, the drugs display very different half-lives, affecting the relative influence of the CYP2C9 polymorphism. The combination approach may have resulted in another outcome compared to if the drugs would have been studied separately in a sufficiently powered study.
- Studies evaluating influence of CYP2D6 polymorphisms on the response to tamoxifen in breast cancer suffered from discrepancies because of analyses of somatic DNA instead of germline

DNA in some studies leading to contradictory conclusions. This has been compounded by studies where different doses of tamoxifen have been combined into the analyses, and lack of distinction of premenopausal and post-menopausal women.

By contrast, the PREDICT-1 trial provides a good example on how to perform these kinds of studies. In total, 1,956 patients from 19 countries revealed a very specific influence of the HLA-B*5701 on the hypersensitivity reactions caused by abacavir. This study was prospective, randomized, designed at elucidating one particular polymorphism and had clear endpoints defined in a well characterized population.

Common and rare genetic variants

Pharmacogenomic testing today is mainly based on methods and approaches determining the more common allelic variants that influence or predict drug response. Recent analyses have revealed that up to 40% of all genetically based interindividual differences in drug PK originate in the distribution of rare mutations in the pharmacogenes in different populations. Furthermore drug metabolism phenotyping in monozygotic and dizygotic twins has shown that only about 40% of the inherited differences in drug metabolism could be explained by the known anticipated gene polymorphisms. The large Next-Generation sequencing (NGS) based consortium efforts such as the 1000 Genomes Project (1000G) and the Exome Sequencing Project (ESP, see *NHLBI Exome Sequencing Project*[1]) have identified the occurrence of less than 18,000 rare genomic variations in genes of importance for control of drug metabolism and transport. The current routine analyses of the common allelic variants may thus not predict the full inter-individual variability in drug PK or PD. The problems of the rare mutations are important since i) these cannot be subject to routine analyses, ii) their functional importance cannot be studied in clinical trials since they are so rare and iii) they together constitute in the specific individual an important reason for inter-individual differences in drug PK and PD. It is however anticipated that genotyping platforms in the future will also encompass known rare mutations, and that guidance regarding the clinical interpretation of the occurrence of these rare mutations will be available. But until then it has to be considered that the individual genotype might not be identified based on the current methods for genotyping and that PK sampling is advisable as a complementary method to reveal the true PK phenotype.

4. Pharmacogenomic variants: phenotyping and genotyping

A genetic polymorphism implies the occurrence of more than one form (or morph) at a frequency above 1% in a population. This definition is of limited use because of the inter-population differences in the distribution of mutations. Furthermore, any interindividual difference in the genotype is often referred to as polymorphisms as well.

Genetic polymorphisms may influence the function of the gene or the abundance of the gene product (gene expression). In many cases the phenotype is not identifiable by genotyping. For example, the identification of CYP2D6 ultrarapid metaboliser (UM) phenotype is difficult since many individuals who are phenotypically UMs do not carry duplications of an active form of the *CYP2D6* gene, which is the only available genetic biomarker for this phenotype. For a true phenotypic classification *in vivo* phenotyping using a probe drug for CYP2D6 like debrisoquine or dextromethorphan must be carried out.

The phenotype with respect to drug PK is often difficult to extrapolate from *in vitro* information regarding metabolism or transport. *In vivo*, the metabolic phenotype may differ from the *in vitro prediction,* because of additional factors such as bioavailability of the probe drug, the overall expression of the enzyme in the liver, hepatic blood flow and the specificity of the enzyme for the

substrate. For example, *in vitro* sertraline metabolism is mediated by several CYP enzymes, however, *in vivo,* only the polymorphism in the *CYP2C19* gene has shown an effect. Hence, for the true identification of the effect phenotyping *in vivo* is important.

The *in vivo* assessment of a functional PK phenotype relies on the use of specific probe drugs. To qualify as a probe substance, a compound must fulfil certain criteria such as i) specificity for the enzyme in question, ii) having a specific, targeted, quantifiable metabolite iii) not being toxic and iv) not interacting with the parent substance or the metabolite from the enzymatic conversion. In the so-called "cocktail approach", several such probe substances are used simultaneously to assess the drug metabolism phenotype of several enzymes. It should be ensured that the substances present in the cocktail do not interact with each other and bias the results. Caution should be taken due to changes in environmental factors. Thus, a phenotype can vary from time to time in the same individual. Such instability in the phenotyping method must be considered when analysing different subjects at different occasions. Furthermore, it is highly recommended only to use cocktails that have been used previously and validated to ensure that there are no DDI problems.

For certain enzymes, the genotype variation can be predictive for the PK phenotype as demonstrated to a great extent by the non-inducible CYP2D6.[2] In this gene more than 110 functionally different alleles have been identified, with their functionality assessed using specific probe drugs *in vivo*. Hence, the *CYP2D6* genotype can be used to predict the metabolic PK phenotype of *in vivo* enzyme activity, whereas other gene products such as CYP2C9, CYP2C19, UGT1A1 and TPMT, have a less robust association between genotype and PK phenotype variation. The lack of a firm link between genotype and phenotype also depends on the rare mutations carried in different individuals. It is therefore recommended that PK-monitoring takes into account all genetic variations present in the subject in question. Clinical validation of genotyping with phenotypes that are assessed *in vivo* are also recommended.

5. The tumour genome

Tumours contain somatic mutations, i.e. differences in the DNA sequence compared to the germline DNA sequence within the same patient. Some mutations will act as driver mutations for tumour development, and are therefore potential drug targets, as well as biomarkers to predict drug response.

Tumour samples obtained by biopsy (or surgical resection) are used for tumour genetic analysis. The quality of the biopsy tissue, as well as location within the tumour is crucial for the correct diagnosis. It is important to ensure in the study protocol that the sample quality of the diagnostic tissue is maximized and the inter-sample differences in quality are minimized (see section *7.2. Analytics*).

Important issues to be considered when analysing the tumour genome include:

Functional heterogeneity

The genetic landscape within a given solid tumour can be substantially heterogeneous. Therefore, it might be challenging to identify the driver mutation if the biomarker is not detected in the tissue sample obtained or if multiple markers are involved. There may also be heterogeneity between primary and metastatic sites, as well as evolution of the tumour genome over time. These aspects must be considered and accounted for when planning clinical studies and when samples from different tumour sites are included in the same study.

The functional effects of genetic variants in cancer may also differ depending on tumour type. For example, inhibition of the BRAF(V600E) oncoprotein by the tyrosine kinase BRAF inhibitor vemurafenib,

is highly effective in the treatment of melanoma. However, in colorectal cancers harbouring the same mutation, response to vemurafenib is very limited.

Liquid biopsy

This involves the isolation of circulating tumour DNA (ctDNA) present in, e.g. plasma, to obtain information about tumour DNA by sequencing the fragments. The liquid biopsy can potentially address challenges relating to functional heterogeneity. This technique may also be useful when a tumour is too small to be visualised for biopsy. Consideration should be given to analysing ctDNA in parallel with tumour tissue DNA whenever possible, in order to explore the utility of this approach in the clinical setting.

Epigenetic modifications of tumour DNA

The expression of different genes is to a great extent governed by methylation (5mC) and hydroxymethylation (5hmC) of certain cytosine residues. The amount of 5hmC is usually significantly decreased in many tumours compared to normal tissues. The commonly used bisulfite technique cannot distinguish between 5mC and 5hmC and in order to evaluate the whole extent of epigenetic modifications methods based on the bisulfite technique in combination with Tet-1 based protocols have to be used. Modification of the extent of cytosine methylation is a common phenomenon seen in tumours causing drug resistance.

The extent of tumour drug resistance can be followed by examination of ctDNA using the liquid biopsy technique. Several studies have linked the presence of altered methylated DNA pieces in plasma to the treatment outcome using the drug. It is advisable to look for recent additions to this field of biomarkers before making a protocol for a clinical trial.

It is important to stress that epigenetic analyses are restricted to the cell type investigated. Because of the high tissue specificity of DNA methylation it is impossible to use blood or any other liquid as a surrogate biomarker for epigenetic changes taking place in different tissues and the epigenetic modifications seen are very specific to different tumours.

6. DNA sequencing design

In designing the genomic sequence analyses it is important to:

- Study relevant genomic variations, particularly those with functional importance
- Employ appropriate methods for DNA isolation that will yield DNA of high quality
- Validate critical sequencing results using, either an independent analytically valid method or resequencing a second amplicon of the same region
- Use published and well curated sequence databases with care and caution
- Employ bioinformatics methods including algorithms of relevance and validate them

Missense, frameshift and nonsense genomic variants, and splicing alterations might be difficult to judge regarding their functional impact. A variety of algorithms such as the PolyPhen-2 and SIFT algorithms are at hand. The underlying concepts of these approaches are mostly similar using sequence conservation metrics that quantify evolutionary conservation. However, they differ in additional attributes such as physiochemical properties, secondary structure, protein domain models or integrated functional residues, and how the results are interpreted. Indeed currently their predictability is not more than 50-70% with respect to the actual phenotypic influence. Further advances to functionally assess detected variants are necessary in order to generate clinically actionable

recommendations. At present it is not advisable to consider available software for prediction of the functional consequences of missense mutations.

Data handling is a major burden after whole genome sequencing (WGS) analyses. As alternatives, bigger genetic analyses can be carried out by whole exome sequencing (WES) or targeted sequencing after DNA capture. The disadvantage using WES or WGS is that sequence data indicating disease risks can appear providing sensitive but for the intended use irrelevant information (incidental findings). Therefore, as an alternative it is advisable to carry out sequencing of genomic regions of particular interest for the drug treatment in question. Specific regions of the genomic DNA are then subjected to next generation sequencing following treatment with capture selection systems designed to answer the relevant pharmacogenomic question. Capture libraries of 5-6 MB will encompass most critical DNA regions believed to influence drug treatment. This strategy is thus of value as incidental findings of disease related genomic variations will be reduced.

7. Quality aspects of pharmacogenomic analyses

7.1. Preanalytics

Pre-analytical variations encompassing (i) sample collection, (ii) labelling, (iii) transport to the site of analysis and (iv) storage before the analytical steps are undertaken, should be minimized by adhering to validated standard operating procedures (SOPs) throughout the workflow (see Draft ICH *Guideline on genomic sampling and management of genomic data E18* - EMA/CHMP/ICH/11623/2016) in order to guarantee highest possible sample quality.

Sample collection (i) varies depending on germline or somatic nature of the pharmacogenomics analyses performed. For germline variation, blood samples will be the simplest method to obtain DNA, whereas buccal swabs or collected saliva may bear the risk for contamination with other than host DNA. For tumour (somatic) genotyping, the quality of the tissue biopsy, as well as the location within the tumour, is crucial for the correct diagnostics (see also section *5. The tumour genome*).

Unambiguous labelling (ii) of the collected samples including patient identifier is crucial, and difficulties arise with coding and anonymisation due to the need to permit withdrawal of consent or follow-up. This could impact research samples stored for subsequent analysis. Utmost attention is required to ensure that clinical procedures during drug development and the period of pharmacovigilance are only undertaken if the identity of the bioprobe is beyond any doubt and certified by a respective authority.

Transport of the sample to the site of analysis (iii) and sample storage (iv) before the analytical steps are undertaken are critical steps in pre-analytics, since the increased sophistication of biomarker analytic procedures, i.e. identification of single nucleotide polymorphisms (SNPs) by the use of arrays, NGS, liquid biopsies, epigenetics etc., demands the use of high quality genomic DNA, which needs to be guaranteed by respective, validated SOPs.

7.2. Analytics

Methods used

A variety of procedures with different technical and/or chemical approaches are currently used for genomic biomarker analytics. The main differences lie in the number of variants tested for identification of the mutations. All the different techniques are in principle useful, as validated by the laboratory standard quality guidelines. It is also recommended that a second, independent (alternative platform) test should be used to validate the results of the genetic analysis. In clinical studies, another means of checking drug exposure such as therapeutic drug monitoring should be utilized. It is

expected that the analysis or the test should provide unambiguous results and that “rare” variants affecting drug safety and efficacy are not excluded (see section *4. Pharmacogenomic variants: phenotyping and genotyping*).

Special caution should be applied when proxy-SNPs are used for predicting the presence of functional relevant SNPs, since there is no 100% linkage between them and sometimes linkage varies greatly between populations, e.g. HLA-A and HLA-B testing by proxy-SNPs. Preference should be given to the direct analysis, i.e. sequencing, of the respective functional relevant SNPs and where proxy or tag-SNPs are used, a risk estimate should be given.

It is important to note that broad sequencing approaches, conventional or NGS, in contrast to techniques focusing on proven functional relevant mutations such as hot-spot-sequencing or pyro-sequencing may reveal many previously unknown mutations. If these are not followed up with studies aimed to identify their potential functional impact, there is a threat of reporting irrelevant/incidental findings. However, algorithms, e.g. PolyPhen-2, SIFT etc., can be used to predict their functional impact. These approaches are mostly similar using sequence conservation metrics, but they differ in which additional attributes such as physiochemical properties, secondary structure, protein domain models and integrated functional residues are used for the interpretation of the results. Currently, the predictability of these algorithms is not more than 50-70% with respect to the actual phenotypic influence and should therefore be used with caution.

NGS specific issues

The reliability of NGS is bound to the coverage the method provides for a specific DNA sequence. For germline genetics, a minimum coverage of 20-50x seems to be a reasonable goal, which, however, depends on the technology as well as the chemistry used. Depending on the sample and allele frequency of a given SNP, those numbers greatly increase for somatic genetics, when using ctDNA, i.e. low allele frequency compared to germline DNA in the central circulation. Irrespective of the type of specimen (germline or somatic), the different analytical steps involved need to be validated in order to provide unambiguous results.

A further challenge for NGS is the analysis of complex loci with high GC-content (guanine-cytosine content) and/or close-by highly homologous genes or pseudogenes resulting in miscalled variants from sequencing artefacts. NGS as opposed to conventional, e.g. Sanger, sequencing relies often on the sequencing of short (couple of hundred base pairs) DNA-reads, therefore limiting the use of NGS when analyzing highly homologous genes, e.g. CYP2D6 and the >95% sequence identical CYP2D7 pseudogene. Promising solutions to these problems include the substantial increase of the “reads”, i.e. up to (and beyond) 1000 base pairs, and/or targeted NGS libraries using sequencing platforms with the latter having the additional advantage of providing phased data sets.

Copy number variations and gene hybrids

CNVs of genes are able to change the phenotype of metabolising enzymes. A well-known example is CYP2D6, where individuals with three or more fully functional copies are defined as UMs. Eliglustat (CERDELGA), a glucosylceramide synthase inhibitor for the long-term treatment of patients with Gaucher disease type 1, is predominantly metabolised by CYP2D6 and its use is not recommended by EMA or U.S. Food and Drug Administration (FDA) in CYP2D6 UMs. Any mislabeling could result in patients being denied crucial treatment or being administered an ineffective drug because of a high metabolism. However, CYP2D6 is also a good example where non thorough analytics could lead to erroneous UM designation. In the case of CYP2D6, it is recognized that this gene may form hybrids (chimeras) with CYP2D7, which shares a staggering 95% identity in the nucleotide sequence with CYP2D6. CYP2D7 seems to be a pseudogene and hybrids, i.e. 2D6/2D7 or 2D7/2D6, between the two

genes are non-functional. Non-functional hybrids bare the additional threat that SNP assays do not discriminate between DNA variations in hybrid and non-hybrid genes.

It is important that CNV assays only evidence functional genes and avoid mislabeling of UMs by sensing non-functional hybrids and that results of SNP assays are not contaminated by those detected in hybrids, therefore delivering unambiguous and correct results.

Allele specificity: Occurrence of mutations in cis and trans

When two different variations with known functional implications are identified in heterozygosity within the same gene, it is important to know whether or not the two variations are on the same allele (in *cis*) or segregated between the two alleles (in *trans*). Therefore, it is imperative that either a risk estimate is given based on historical data about the chance of both SNPs being on the same allele or, preferably, an analytics is used that can give unambiguous information of the specific allele location of the SNPs.

Liquid biopsies: Circulating tumour DNA analysis

The cardinal challenge for circulating somatic biomarker analytics is the presence of a vast background of germline genetic 'contamination'. Nucleus bearing cells in the blood carry the germline genome with the important exception of haematological malignancies. When liquid biopsies are used, efforts should be in place to ensure separation of germline and somatic DNA. The use of digital-PCR or NGS, however, does have the potential to provide the sensitivity needed for performing circulating DNA analytics.

Intra patient verification of genotyping results

Most analytical procedures are based on the direct or indirect use of priming nucleic acid sequences. The binding of these primers and/or probes may be hampered if the sample tested shows mutations within the primer or the probe-binding sequence. Additionally, it is known that DNA quality as well as the chemistry used, e.g. DNA polymerases, can hamper the analytics for a given patient and therefore deliver an ambiguous result. The best strategy to avoid this is the employment of two independent (chemistry/technology) approaches in the very same patient, i.e. intra patient verification (IPV). Only when the two results of the IPV are identical, the results should be used. This applies to PGx analytics during drug development as well as for pharmacovigilance.

Reporting the genetic call

The nomenclature of the biomarker calls is heterogeneous in form and content. Whereas some laboratories identify the variation by the nucleotide position in the genomic DNA, others give the position in the cDNA and again others give the change on the amino acid level. The latter of course does not apply for mutations within non-coding regions. In the case of variations that are positioned within the coding region/protein, the nomenclature is usually straightforward and not prone to many mistakes. However, variants that are defined with genomic DNA coordinates can vary greatly depending on the reference sequence used. As such, it is important to provide information regarding the reference sequence in the report.

The nomenclature system for the CYP star (*) allele designation takes into account mutations causing functional consequences such as stop mutations or amino acid changes. The star-number is unique for the presence of one or more functionally important mutations on the same allele and each *-marked allele has its phenotypic description based on the functionally important mutations present (see *Human Cytochrome P450 (CYP) Allele Nomenclature website*[2]). However, as a bigger population is tested, more haplotypes for a designated (*) allele appear, making an unambiguous nomenclature by (*) alleles challenging.

A good laboratory report from genetic analyses should indicate what was measured, i.e. the gene and the rs-number of the SNP(s), identified SNPs including the respective rs-numbers, the interpretation of the SNPs to alleles, e.g. CYP2D6 *4/*6, a description of the functional implications of these alleles or SNPs, i.e. 2 non-functional alleles in the case of CYP2D6 *4/*6, and a prediction of the phenotype based on the found SNPs of the corresponding gene, e.g. extensive metaboliser, extensive/intermediate metabolisers etc.

Accreditation of PGx analytics

Not all European Union member states require accreditation by a public authority for performing PGx analytics. However, PGx implies the use, the termination and/or the changing of the dose of a specific drug—all measures that require meticulous analytics leading to an unambiguous genetic call in order to influence decision making. Therefore, appropriate oversight is necessary and this may require accreditation in the different member states or be subjected to validation using nationally accepted procedures for predictive biomarker analytics, including intra-laboratory proficiency testing. ISO15189 certification or corresponding certificates, e.g. American standards like College of American Pathologists (CAP) guidance and the Clinical Laboratory Improvement Amendments (CLIA), would be important in order to harmonize standards of good laboratory practice.

7.3. Sample repository for retrospective studies

Several national and European initiatives led to the establishment of DNA-repositories, with a very broad scope and access. Often there are retrospective genomic analyses in many studies and clinical trials. For retrospective analyses of such samples for PGx, the EMA *Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products* (EMA/CHMP/37646/2009) provides detailed information. As described in this guideline, increasingly sophisticated genomic techniques are being employed, requiring the establishment of dedicated PGx-sample-repositories employing scrupulous standards governing sample quality and usage. This would ensure that retrospective PGx related studies using DNA analyses, including NGS, and epigenetic investigations can be performed on the stored samples and are not limited by their quality and/or amount.

8. Study design

The aspects of genomic variation have to be considered in the design of clinical trials and is of critical importance for a successful outcome.

Clinical validation of a genomic biomarker is usually required in order to confirm association with a functional phenotype important for clinical pharmacokinetics, efficacy or safety. Good pharmacogenomic practice will impose certain requirements to the study design that are necessary to allow the validation of genomic biomarkers for clinical use or for drug development.

The chosen biomaterial should be appropriate for the study objective. It is important to ensure in the study design that the quality of the diagnostic tissue is maximized and the inter-sample differences in quality are minimized (see section *7.1. Preanalytics).*

A predictive genomic biomarker may be a single marker or be comprised of a multi-marker signature or algorithm. If possible the relative importance of the functional contribution of each component to the predictive value of the genomic biomarker should be defined. Predictive genomic biomarkers may be binary (e.g. gene mutations) or be described by classifiers (e.g. gene expression levels, loss of heterozygosity). In some cases, the genomic biomarker also serves as the molecular drug target (e.g. Her-2 receptor and trastuzumab).

8.1. Exploratory development of genomic biomarkers

Predictive genomic biomarkers may be identified for the first time during early exploratory studies, or evaluated during early exploratory studies based on biological plausibility or non-clinical research. Significant unexplained inter-patient variability in response (outliers) at any stage of clinical development warrants investigation to identify a possible genomic biomarker association. Information relating to the genomic biomarker may also arise from previous observations on other drugs with shared characteristics, e.g. substrates for CYP2D6.

Early phase studies are often single arm cohort studies, in which case it may not be possible to establish the sensitivity, specificity and predictive value of the genomic biomarker. Selection bias, related to the availability of biological samples from all subjects, or failure connected to the assay in question, should be minimized where possible. Selection bias may be a particular issue with case-control studies, particularly those relying on retrospective patient recruitment or retrospective analyses. In the case of genome wide association studies (GWAS), due to multiplicity issues, only very strong associations are likely to be reproducible during subsequent development.

Novel trial designs, including umbrella and basket studies, and adaptive designs, are being increasingly proposed, particularly in oncology drug development. Importantly, they allow for a more patient-centric approach. At present, there is limited regulatory experience, and early dialogue is advised if these designs are to be used in drug development programmes.

Acceptable analytical validity of the assay should be demonstrated as early as possible during clinical development, so that early clinical findings can be considered relevant to later clinical development.

8.2. Confirmatory development

Confirmation of the clinical validity of a predictive genomic biomarker would normally be undertaken when a genomic biomarker has shown sufficient promise during exploratory development. This involves replication of the findings in independent cohorts. The study design is to some extent dependent on the outcome being evaluated. Thus, for a genomic biomarker that might predict a rare severe adverse drug reaction, a case control design may be more applicable than a prospective RCT.

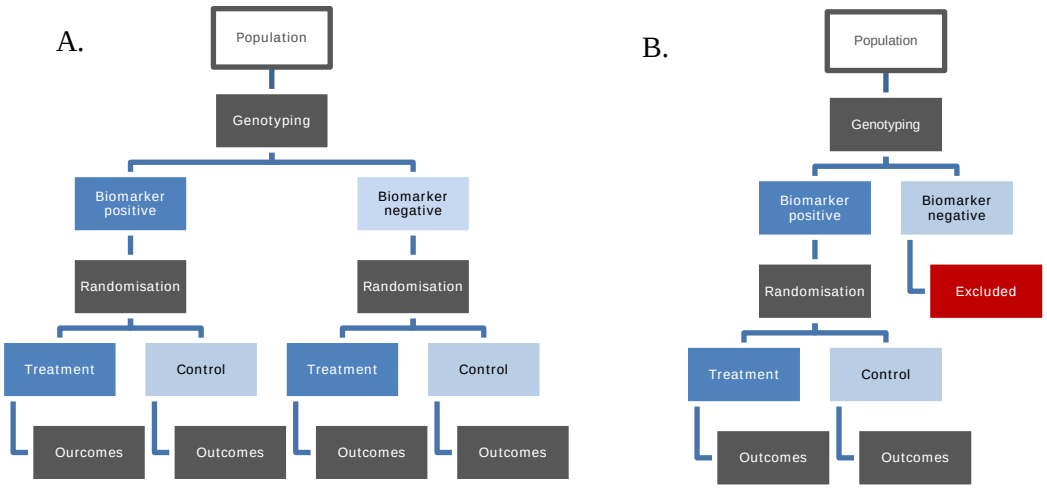

Figure 1. Randomized controlled trial designs for testing pharmacogenomic biomarkers. A , unselected RCT. B, enriched RCT.

It is critical that analytical validity of the assay has been established, and that the risk of assay failure is minimised, for clinical validity to be confirmed. In the case of classifiers, the cut-off point for

determining the genomic biomarker status should have been established based on robust evidence, including evaluation of receiver operating characteristic (ROC) curves, where applicable.

An **unselected** RCT, in which eligibility is not based on genomic biomarker status, is the preferred design for clinical validation of a predictive genomic biomarker evaluating drug efficacy or for the prevention of a common adverse drug reaction (see Figure 1.A). The inclusion of a control arm and stratification of randomisation by genomic biomarker status (i.e. genomic biomarker positive or genomic biomarker negative) will permit estimation of sensitivity, specificity, and predictive value. It will be important to consider whether the biomarker-dependent response, or the response in the overall group, will be the primary analysis, depending on the objectives. The required sample size may be relatively large, compared to other trial designs. The risk of multiple comparisons needs to be accounted for.

Retrospective confirmation of the validity of the biomarker might be possible using data from one or more well-conducted RCTs. This approach would require knowledge of genomic biomarker status from majority of patients (to avoid selection bias), and the documentation of the analysis plan before evaluation.

In an **enriched design**, eligibility is informed by genomic biomarker status (see Figure 1.B). Strong biological plausibility linking the genomic biomarker and the disease, and persuasive preliminary evidence of association between the genomic biomarker and drug response are needed. Enriched designs are most applicable when the genomic biomarker either forms or influences the therapeutic (drug) target directly. The regulatory acceptance of excluding genomic biomarker negative patients will depend on the strength of evidence provided for the lack of effect in these patients.

8.3. Considerations for dose selection

If a certain genotype bears the risk of adverse drug effects due to increased exposure, it may be appropriate to apply a stratified dosing scheme for the genotype groups. Dosing strategies need to be appropriately refined for genotype-guided studies taking into account the current regimens being used in the healthcare setting, and the availability of different dose formulations and the pharmacological characteristics of the drug. Alternatively, if it is known that patients with a certain genotype will not profit from a particular therapy, it may be appropriate to choose a study design involving a different drug regimen to patients that are carriers of the genotype at risk. Good pharmacogenomic practice emphasizes the need to consider the best knowledge of the phenotype and the clinical consequences of therapy within a genotype group for the design of an appropriate trial.

9. Pharmacogenomic biomarkers and translation into the clinics today

Sometimes, genotypes are strongly associated with a clinical phenotype, and the mechanisms of the association at the molecular level are complex, and not necessarily dependent solely on the concentration of the drug. In these cases, phenotyping by measurement of drug concentrations is of no help, and the clinical symptoms represent the phenotype. This has been observed with serious immune-mediated adverse drug reactions, where strong associations with HLA have been demonstrated. This has led to pre-emptive pharmacogenomic testing. For example HLA genotyping is used for the prevention of abacavir hypersensitivity syndrome, which comprises fever, rash, gastrointestinal tract and respiratory symptoms that become more severe with continued dosing, and can become life-threatening. The HLA allele *B*57:01* is associated with abacavir hypersensitivity with a negative predictive value of 100% in immunologically confirmed cases which led to recommendations

for pre-emptive screening of *HLA-B*57:01* and warnings from FDA, EMA and the national authorities in Europe. Similarly, carbamazepine can lead to Stevens-Johnson Syndrome and toxic epidermal necrolysis, conditions associated with 10% and 30% mortality respectively. In certain ethnic groups of South East Asian origin (Han Chinese, Thai and Malays), *HLA-B*15:02* is an important predisposing allele with a negative predictive value of 100%. Again, the summary of product characteristics now includes a recommendation to genotype patients of certain ethnic groups for this HLA allele prior to prescribing carbamazepine.

In such cases, there is no other phenotype measurement or predictive phenotype (apart from clinical symptoms) available that can be assessed to measure the function of the genetic variant in HLA. Since 2000, about 24 different HLA allele associations have been identified with differing predictive values, different phenotypes (skin, liver, bone marrow and muscle injury) and in different ethnic groups. It is anticipated that many of these could provide useful genomic biomarkers in the future. The clinical validity of these associations has been confirmed through various clinical study designs including RCTs, cohort studies and case-control studies. An important aspect of clinical validation is through replication by independent groups in different patient populations. It is unlikely that such serious adverse reactions will be frequent enough during drug development to warrant HLA testing, but are often identified in the post-marketing setting.

As with other types of pharmacogenomic biomarkers, different methods can be used for HLA typing, ranging from immunological serotyping to next generation sequencing. However, given the complexity of the HLA region, it is important that any typing is undertaken to 4 digits, as 2-digit typing will not distinguish closely related alleles which have different risk profiles for such adverse reactions. For example, *HLA-B*57:01* predisposes to abacavir hypersensitivity, while *HLA-B*57:03* does not.

10. Future dynamics of drug labels

The Summary of Product Characteristics (SmPC) or the label sets out key elements of drug benefits and risks relevant to the clinical use of the product defined during the medicine regulatory assessment process. At present, the SmPCs of about 150 drugs approved by the European Commission (EC) mainly used in oncology include pharmacogenomic information and for the US FDA, a similar number of drug labels apply (see FDA *Table of Pharmacogenomic Biomarkers in Drug Labeling*[3]). Furthermore, pharmacogenomic labels are of particular importance for treatments where the therapeutic index is narrow and thus where a small over-dosing poses a substantially increased risk for adverse drug reactions.

Where possible, the SmPC should inform on important inter-individual variability in drug pharmacokinetics or response, and, to which extent, such variability may have a genetic basis.

Recommendations on how to include pharmacogenomic information in the SmPC is provided in the EC SmPC guideline, the EMA *Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products* (EMA/CHMP/37646/2009) and the *Guideline on key aspects for the use of pharmacogenomics in the pharmacovigilance of medicinal products* (EMA/CHMP/281371/2013).

Current SmPCs often contain pharmacogenomic data available at time of the initial registration of the medicinal product. However, for adequate pharmacogenomic information to be maintained or improved, it is essential that relevant pharmacogenomic data gathered in the post-registration phase is used to update the SmPC as and when necessary during the life cycle of a product. It is recommended that the Marketing Authorisation Holders should aim for providing relevant and up-to-date

pharmacogenomic information, thereby facilitating the appropriate use of pharmacogenomic information by prescribers and patients. Such updates of the SmPC may be initiated by the Marketing Authorisation Holders' post-registration studies or information arising from other studies.

Abbreviations

1000G	1000 Genomes Project
5hmC	5-hydroxymethylcytosine
5mC	5-methylcytosine
CAP	College of American Pathologists
CIOMS	Council for International Organizations of Medical Sciences
CLIA	Clinical Laboratory Improvement Amendments
CNV	Copy Number Variation
ctDNA	Circulating Tumour DNA
CYP	Cytochrome P450 Enzyme
DDI	Drug-Drug-Interaction
DNA	Deoxyribonucleic Acid
EC	European Commission
EMA	European Medicines Agency
ESP	Exome Sequencing Project
FDA	U.S. Food and Drug Administration
GWAS	Genome Wide Association Study
HLA	Human Leukocyte Antigen
ICH	International Council for Harmonisation
IPV	Intra Patient Verification
miRNA	microRNA
NGS	Next-Generation Sequencing
PCR	Polymerase Chain Reaction
PD	Pharmacodynamics
PGx	Pharmacogenomics
PK	Pharmacokinetics
RCT	Randomized Controlled Trial
RNA	Ribonucleic Acid
ROC-curve	Receiver Operating Characteristic Curve
SmPC	Summary of Product Characteristics
SNP	Single Nucleotide Polymorphism
SNV	Single Nucleotide Variation

SOP	Standard Operating Procedure
UM	Ultrarapid Metaboliser
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing

Definitions

Allele	one of a number of alternative forms of the same gene or the same genetic locus
Allele specific SNP analytics	determining on which allele (mother or father) a certain genetic variation is located
Analytical specificity	the ability to unequivocally assess the target nucleic acid in the presence of other nucleic acids or other components, which may be expected to be present
Analytical sensitivity	the detection limit, which is the lowest amount of nucleic acid, which can be specifically detected by a pharmacogenomic assay
Biomarker	a characteristic that is measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacological responses to a therapeutic intervention
Clinical sensitivity	the proportion of individuals with a specified clinical disorder or clinical effect whose test values indicate that the disorder or clinical effect is present (e.g. the mutation associated with the disorder is identified)
Clinical specificity	the proportion of individuals who do not have a specified clinical disorder or effect and whose test results indicate that the disorder or clinical effect is not present
Epigenetics	changes to the genome that do not involve a change in the nucleotide sequence, e.g., DNA methylation or histone modification
Functional 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
Genomic biomarker	a measurable DNA and/or RNA characteristic that is an indicator of normal biologic processes, pathogenic processes, and/or response to therapeutic or other interventions (ICH15)
Genetic biomarker	a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species; a genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change, or a long one, like mini satellites

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
Genotype	a specific form of a gene
Germline DNA	the DNA in germ cells (egg and sperm cells that join to form an embryo); germline DNA is the source of DNA for all other cells in the body. Also called constitutional DNA
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
Pharmacogenetics	the study of variations in DNA sequence as related to drug response, a subset of pharmacogenomics (ICH15); the study of interindividual variations in DNA sequence related to drug disposition (pharmacokinetics) or drug action (pharmacodynamics) that can influence clinical response (CIOMS VII (2005))
Pharmacogenomics	the study of variations of DNA and RNA characteristics as related to drug response (ICH15); the application of genomic technologies to elucidate disease susceptibility, drug discovery, pharmacological function, drug disposition and therapeutic response (CIOMS VII (2005))
Phenotype	observable characteristics influenced by genotype; may be influenced by other additional factors, e.g., the environment
Polymorphism	occurrence of more than one form (or morph) of a (functional) phenotype in a frequency that is stable in different populations, and a frequency above 1%
Somatic mutation	genetic variation that can be passed to the offspring of the mutated cell; Somatic mutations in contrast to germline mutations, are inherited genetic variations that happen in the germ cells (i.e., sperm and eggs)

References

1 NHLBI Exome Sequencing Project: http://evs.gs.washington.edu/EVS/

2 Human Cytochrome P450 (CYP) Allele Nomenclature websit: http://www.cypalleles.ki.se/criteria.htm

3 FDA Table of Pharmacogenomic Biomarkers in Drug Labelling: http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm