Second International ESPT Meeting
Lisbon, Portugal, 26–28 September 2013

The second international European Society of Pharmacogenomics and Theranostics (ESPT) conference was organized in Lisbon, Portugal, and attracted 250 participants from 37 different countries. The participants could listen to 50 oral presentations, participate in five lunch symposia and were able to view 83 posters and an exhibition. The first part of this Conference Scene will focus on the pharmacogenomics and biomarkers used in medical oncology, and in particular solid tumors. In addition, this article covers the two keynote conference introductory lectures by Ann K Daly and Magnus Ingelman-Sundberg. The second part of this article will discuss the clinical implementation of pharmacogenomic tests; the role of transports and pharmacogenomics; how stem cells and other new tools are helping the development of pharmacogenomics and drug discovery; and an update on the clinical translation of pharmacogenomics to personalized medicine. Part two of this Conference Scene will be featured in the next issue of Pharmacogenomics.

The meeting was organized by the European Society of Pharmacogenomics and Theranostics (ESPT) board members under the chairmanship of its president supported with the local help of CarolinoMonteiro, Sara Torgal, Luis Silva Santos and Pedro Gil, as well as help from the ESPT office in Nancy, France, Laurence Ben Rezig, the UMR INSERM U.1122 research team and Benjamin Richier for Com & Co, Marseille, France.

The meeting was held under the auspices of International and National Societies which were all represented at the opening ceremony (Figure 1): International Federation of Clinical Chemistry and Laboratory Medicine (IFCC); International Union of Basic and Clinical Pharmacology (IUPHAR); European Federation of Clinical Chemistry and Laboratory Medicine (EFLM); The Federation of European Pharmacological Societies (EPHAR); European Federation of Clinical Chemistry and Laboratory Medicine (EFLM); The Federation of European Pharmacological Societies (EPHAR); European Society of Predictive Medicine (EUSPM); European Society for Biopreservation and Biobanking (ESBB); Portuguese Society of Pharmacology (SPF); Portuguese Society of Clinical Chemistry (SPQC); Portuguese Association of Students of Pharmacy (APEF); Spanish Society of Clinical Biochemistry and Molecular Pathology (SEQC); Spanish Society of Pharmacogenetics and Pharmacogenomics (SEFF); Spanish Society of Clinical Pharmacology (SEFC); Ordemdos Farmacêuticos (OF); Ordemdos Médicos (OM); Ordemdos Biólogos (OB).

Five lunch symposia were also organized, as outlined below:

**Randox**
- Application of Biochip array technology for multiplex pharmacogenomics profiling, John Lamont
- The ‘Rheumastrat’ biochip array; a multiplex SNP array for the stratification of therapy response in rheumatoid arthritis patients, Cathy McGeough
- The importance of VEGF in pharmacogenomic, Sophie Visvikis-Siest

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10.2217/PGS.14.35 © 2014 Future Medicine Ltd
Pharmacogenomics (2014) 15(5), 593–599
ISSN 1462-2416
**Potential to enhance colorectal cancer patient pathways through multiplex mutational profiling of KRAS, BRAF and PIK3CA genes, Helena Murray**

**Affymetrix**

- Right drug, right patient, right dose, right time: The translation of pharmacogenetics into practice, Johanne McGregor
- Development of pharmacogenetic guidelines by the Dutch pharmacogenetics Working Group, Jesse J Swen

**Life Technologies**

- Your new research companion for Pharmacogenomics, Dominique Dewolf
- Design and validation of a SNP Panel in the QuantStudio™ OpenArray® Platform for Pharmacogenetic Research in Psychiatry, Miquel Tuson
- Ion-Torrent Next-Generation Sequencing for cancer mutation detection, Marivi Carretero

**Qiagen – Applications of molecular screening**

- A new IDH1/2 PCR assay for one-step detection of 12 IDH1 and IDH2 mutations in glioma, Hélène Peyro-Saint-Paul
- From sample to insight: bringing next-generation sequencing into clinical routine, Fabienne Hermitte

**Agilent**

- Next Generation Thinking – Integrated Genomics Solutions for Human Disease Research, Didier Goidin

**Advanced training course**

An Advanced Training Course with six speakers was organized the day before the main meeting: attendees also participate in the full meeting. Two introductory lectures opened the meeting, as described below.

**Opening lectures**

The first opening lecture was presented by Ann K Daly (Newcastle University, Newcastle, UK) with a lecture entitled ‘Recent Progress in Molecular Methods for Pharmacogenomics Studies’. She presented three groups of pharmacogenomics applications, and compared genotyping and phenotyping for TPMT deficiency screening in treating patients for cancer and for CYP2C19 genotyping by a new point-of-care test for clopidogrel. Furthermore, she focused on warfarin pharmacogenomics, with the genome-wide association studies (GWAS) confirming data from candidate gene studies. A third topic was the involvement of HLA in idiosyncratic adverse drug reactions – that is, for flucloxacilline and amoxicillin. Concluding, Prof. Daly addressed new technologies, with the exome analysis seeming to identify new genes involved in adverse reactions.

Figure 1. Opening session with the official representatives.
Magnus Ingelman-Sundberg (Karolinska Institute, Stockholm, Sweden) presented the second lecture on ‘Pharmacogenomics of Endogenous Metabolism and its Implication for Behavior, Psychopathology and Treatment’. After an introduction on the different GWAS looking for drug-metabolizing enzymes (DME), he discussed epigenetic data on hepatic DME genes with DNA methylation landscapes. Only a small part of DME genes show differences in methylation, including promoter regions. The methylated amplicons are differently regulated and are at the origin of expression variations between fetal liver and adult liver, that is, for GST, ABC genes. Hydroxymethylations (5-hydroxymethylcytosine) are in the enhancers and in CpG islands and not in the intergenic regions. Interestingly, in the liver 30–40% of cytosine modifications are 5hmC, indicating that previous work using the bisulfite technique has missed the important distinction between the more inhibitory 5mC and the more activatory 5hmC in the liver. He showed how 3D cultures of hepatocytes are a better tool for studying drug-induced liver toxicity, with more expression of relevant genes.

However, the highlight of the presentation was the discussion of CYP2C19 expression in the brain of mice and the effect of differential expression on the formation of the hippocampus. The mouse model is bringing new hypotheses on the potential role of CYP2C19 in brain function. CYP2C19 was expressed in the fetal but not adult brain. The differences in the transgenic model compared with the adult type were that the hippocampus area decreased, whereas the behavior tests showed a greater stress, an increased activity and an increased anxiety. The molecular background seems to be a decreased number of immature neurons in the dentate gyrus. This demonstrates that CYP2C19 could have a future potential role in brain disorders.

First session: pharmacogenomics & biomarkers in medical oncology: across the spectrum of solid tumors

Bohuslav Melichar (Olomouc, Czech Republic) & Rui Medeiros (Porto, Portugal)

Despite the advent of new therapies, cancer currently represents one of the most important public health problems. After decades of indiscriminate use of anticancer drugs, more personalized approaches have emerged that made the concept of targeting the right agent for the right patient a reality. Across the spectrum of different primary tumors, various strategies have evolved to predict the pharmacokinetics, toxicity and, most importantly, the activity of anticancer drugs. Although the topics discussed represent only a fraction of problems under active research, the spectrum of tumors and therapeutic approaches illustrates the possibility of universal utilization of pharmacogenetic and theranostic approaches in the pharmacotherapy of cancer.

Nucleoside analogues, including 5-fluorouracil or gemcitabine, represent the backbone of chemotherapy regimens used in the treatment of gastrointestinal malignancies. Although these agents are, in general, well tolerated, the instances of serious, sometimes even fatal complications represent a major issue in the management of patients with gastrointestinal cancers. The topic of predicting toxicity of 5-fluorouracil and gemcitabine and, most importantly, integrating the predictive testing into routine practice was introduced by J Ciccolini (Aix-Marseille University & CHU Timone, Marseille, France). Genetic polymorphisms of dihydropyrimidine dehydrogenase are responsible for more than 50% of cases of severe toxicity associated with the administration of fluoropyrimidines. In addition, there are clinical data demonstrating that the 5-fluorouracil dose adjustment based on dihydropyrimidine dehydrogenase activity results in reduction of toxicity and improved treatment delivery. However, the lack of standardization of the methods to assess dihydropyrimidine dehydrogenase activity has so far represented an obstacle in the widespread adoption of dihydropyrimidine dehydrogenase testing for all patients scheduled to be treated with fluoropyrimidine-based chemotherapy. Cytidine deaminase is the key enzyme responsible for gemcitabine elimination. Because of the unclear relationship between the genotype and phenotype, the measurement of enzymatic activity is used to assess cytidine deaminase status. It has been demonstrated that decreased cytidine deaminase activity is associated with toxicity in
patients treated with gemcitabine. Interestingly, high cytidine deaminase activity is associated with toxicity of another anticancer drug, capecitabine, a fluoropyrimidine. Toxicity after administration of capecitabine has also been described in association with the polymorphism of the thymidylate synthase gene. The routine assessment of the genetic polymorphisms or activities of these enzymes would not only prevent most cases of severe toxicity, but would also result in better delivery of anticancer drugs using individually tailored dosing.

Lung cancer is the leading cause of cancer mortality in the EU. The targeted therapeutic approaches that emerged in patients affected with this tumor during the past decade have stressed the importance of predictive biomarkers. This topic was addressed by G Berchem (Luxembourg Hospital Center, Luxembourg). Mutations of the \( \text{EGFR} \) gene predict the sensitivity of tumor to the low-molecular-weight EGFR tyrosine kinase inhibitors gefinitib and erlotinib. Moreover, another targeted approach in non-small-cell lung cancer involves anaplastic lymphoma kinase inhibition using crizotinib. The identification of molecular biomarkers is crucial as these drugs are active only in a minority of patients, and indiscriminate use of these novel agents would not only mean wasting of resources, but, more importantly, wasting precious time for effective therapy while using an agent that cannot work in a given patient.

The association between glutathione S-transferase gene polymorphism, busulfan metabolism and therapeutic outcome was discussed by M Ansari (CANSEARCH, Foundation for Children’s Cancer, Geneva, Switzerland). Busulfan is the principal component of myeloablative regimens used in pediatric patients undergoing hematopoietic stem cell transplantation. Marked interindividual differences in busulfan pharmacokinetics may result in complications or treatment failure. Glutathione S-transferase is the principal enzyme responsible for busulfan elimination. It has been demonstrated that polymorphisms in the genes coding for glutathione S-transferase isoforms may partly explain the pharmacokinetic variability after intravenous administration of busulfan.

Colorectal carcinoma is one of the most common tumors and one of the leading causes of mortality in the EU. Despite efforts aimed at screening, the tumors are still diagnosed late. In addition, distant metastases subsequently manifest in a significant proportion of patients treated for early colorectal carcinoma. Despite the advent of targeted agents, cure is not possible in most cases of metastatic colorectal carcinoma. Cytotoxic chemotherapy remains the main component of systemic therapy. However, in contrast to other tumors, only a limited spectrum of cytotoxic drugs has reproducible activity in colorectal carcinoma. Historically, many research efforts have been aimed at compensating the limited number of active agents available in colorectal carcinoma by identifying elaborate regimens of utilization of these drugs. The benefits of hepatic arterial infusion, prolonged continuous infusion, or administration using chronomodulated schemes have been explored. D Paez (Santa Creu i Sant Pau Hospital, Barcelona, Spain) presented another approach using pharmacodynamic parameters in defining the dose of irinotecan in patients with metastatic colorectal carcinoma. Gastrointestinal toxicity (diarrhea) is the dose-limiting toxicity of irinotecan. In clinical practice, gastrointestinal toxicity may become life-threatening when associated with myelosuppression. Uridine diphosphate glucuronosyltransferase is the key enzyme responsible for elimination of 7-ethyl-10-hydroxycamptothecin (SN-38), the active metabolite of irinotecan. Genetic polymorphism of uridine diphosphate glucuronosyltransferase is reflected in marked interindividual differences of pharmacokinetics of SN-38 that also result in marked differences in toxicity. A prospective study has demonstrated that, in fact, the majority of subjects receiving irinotecan are significantly under-dosed, with the maximum tolerated dose being more than twice higher than the currently recommended dose, while in a minority of patients with phenotype that results in slower SN-38 metabolism the maximum tolerated dose is 30% lower than currently recommended. Moreover, higher irinotecan dose resulted in a significantly increased response rate and in prolongation of time to disease progression. Given
the limited number of active agents available for the management of metastatic colorectal carcinoma, pharmacogenetic-based dosing may be regarded as a major step forward.

Pancreatic cancer remains the tumor with probably the most dismal prognosis of all malignancies. The disease is diagnosed late in most cases and sensitivity to systemic chemotherapy is limited. In fact, the administration of currently available agents results only in a modest prolongation of overall survival, while being associated with significant, sometimes even life-threatening, toxicity. One of the reasons behind the limited sensitivity or even primary resistance of pancreatic carcinoma is the lack of expression of molecules mediating transport of the drug into the cell, or even the presence of transporters mediating the elimination of cytotoxic drugs or metabolites from the cytoplasm. The research on the effect of the presence of membrane transport molecules on the outcome of systemic treatment in patients with pancreatic carcinoma was summarized by B Mohelnikova-Duchonova (National Institute of Public Health, Praha, Czech Republic). It was demonstrated that the expression of ABC efflux transporters is increased and the expression of SLC transporters responsible for anticancer drug uptake is decreased in pancreatic cancer cells. Moreover, a significant association of SLC polymorphism with the overall survival was evident even in patient cohorts of limited size. Still, the research on these biomarkers that might predict pancreatic cancer chemosensitivity is at the very beginning, and in many instances the data are inconclusive, or conflicting results have even been reported by different authors.

The incidence of prostate cancer increases with age, and with aging of the population in the EU, prostate cancer is becoming a major public health problem. The topic of pharmacogenomics of castration-resistant prostate cancer was introduced by R Medeiros (Instituto Portugues de Oncologia, Porto, Portugal). Hormonal castration therapy (HCT) has been the standard of care in advanced and metastatic prostate cancer. The proposed rationale is that androgen deprivation inhibits the proliferation of prostate tumoral cells leading to tumor regression. However, although patients benefit from the treatment, the inevitable progression will occur with a decrease in the mean overall survival. The establishment of new biomarkers to predict response to this treatment remains to be determined, and the molecular mechanisms involved in acquisition of resistance to hormonal castration have not been clarified. Genetic polymorphisms associated with prostate cancer aggressiveness may also be implicated in the process of resistance to hormonal castration. Several lines of evidence indicate that molecules implicated in androgen pathways are liable to prostate cancer refractoriness to hormonal castration. Therefore, functional polymorphisms in genes related to this pathway may impact androgen bioavailability and help predict resistance to hormonal castration. Previous studies on the androgen pathway found association between CYP19A1, HSD3B1 and HSD17B4 genetic variants with the efficacy of HCT. Recently, multivariate analysis showed an increased risk of developing resistance to hormonal castration under the influence of SHBG genetic polymorphism. Furthermore, to address the complexity of these mechanisms, with the involvement of several probably interconnected pathways, it has been also reported that patients under HCT and carrying the HIF1A +1772 T-allele (rs11549465) present an increased risk for developing distant metastasis and an independent sixfold increased risk for resistance to HCT. The acquisition of castration-resistant phenotype is also associated with the activation of signaling pathways mediated by growth factors. TGFβ1 and its receptors have an important role in tumor progression, being the pro-apoptotic function modulated by the expression of TGFBR2. Within this line of research, it was found that TGFBR2-875GG homozygous patients present lower expression levels of TGFBR2 mRNA. Furthermore, in these individuals, carriers of the GG genotype, a link was found with higher Gleason grade and the increased risk of an early relapse after HCT. The TGFBR2-875G>A contribution to an early relapse in HCT patients, due to changes in mRNA expression, supports the involvement of TGFβ1 pathway in CRPC.

We can conclude that there is now an increasing body of evidence to support the use of pharmacogenomic approaches
in the management of patients with a wide range of malignant disorders, not only to identify patients at increased risk of side effects, but also to guide the selection of the right drug for the right patient.

After this session, four oral presentations described other pharmacogenomics tools and recommendations. Charles Cantor (Sequenom, San Diego, USA), in his presentation entitled 'Personalized Pharmacogenetics', suggested the use of tumor-specific genetic and epigenetic biomarkers detectable in tissue biopsies and in plasma for the monitoring of cancer therapy and patient management. The use of DNA Mass Spectrometry was proposed as a sensitive, quantitative and cost-effective way of mutation identification and its application in the Sequenom Absorption, Distribution, Metabolism and Excretion (ADME) Pharmacogenetics Panel Genes was presented (it includes 192 assays in 36 genes). Furthermore, a novel approach for multiplex ultrasensitive detection assay of somatic mutations in tumor samples has been developed and it presents a high analytical sensitivity (93%) and specificity (99%). A preliminary panel of somatic mutations is already constructed. The use of Onco-Carta™ panel was also proposed for the assessment of the tumor clonality, where tumor-specific mutations are described. Finally, an interesting application of plasma sequencing for therapy monitoring of cancer patients was presented. Malin Lindqvist Appell (Linköping University, Sweden), in the presentation entitled 'Formation of a TPMT Nomenclature Committee – Bringing Order and Consensus Around Known and Novel TPMT Sequence Variants', discussed the problem of nonstandard clinical nomenclature for the genetic variants of the DME TPMT. The related gene is polymorphic and many sequence variants cause a decreased enzyme activity. As TPMT metabolizes the thiopurines 6-mercaptopurine, 6-thioguanine and azathioprine, the pretreatment determination of TPMT genetic status is suggested in many countries for the dose optimization before the initiation of thiopurine treatment. To avoid the confusion, a panel of 16 scientists around the world has created a nomenclature committee to define nomenclature and numbering of novel variants for the TPMT gene. The available website was described during this talk. The other two presentations presented the results of research studies that could have some clinical implementation on pharmacogenomics. Maria Stathopoulou (University of Lorraine, Nancy, France) presented a work entitled 'Synergy Between Clopidogrel and Calcium-Channel Blockers for Blood Pressure Regulation Possibly Involves CYP2C19 Polymorphism'. In this study, a synergistic effect between clopidogrel and calcium-channel blockers on blood pressure levels was observed in a sample of 2322 patients with acute myocardial infarction or unstable angina. This synergy was protective as it was associated with significantly lower levels of diastolic blood pressure. Additionally, the effect of the CYP2C19*2 polymorphism (rs4244285), which is a known determinant of clopidogrel’s activity, was also assessed in terms of blood pressure level modification in a healthy population of 2155 individuals. A significant lowering effect for both systolic and diastolic blood pressure was revealed for the carriers of the minor allele, thus indicating a possible link between clopidogrel and blood pressure regulation. Finally, Nádia Marques Grilo (NOVA University of Lisbon, Portugal), in her presentation 'Relevance of CYP2C19 Genotypes in Nevirapine Biotransformation', described a study on the assessment of the influence of CYP2C19 variant alleles (*2 and *17) on nevirapine (NVP)/12-OH-NVP metabolic ratio. The NVP biotransformation was studied in a population of 51 HIV-infected patients who were in NVP-based combined antiretroviral therapy (400 mg) for more than 1 month. Significant differences were observed only between NVP concentrations and carriers of the CYP2C19*2, thus suggesting that this variant plays a role in NVP biotransformation and the underlying severe idiosyncratic hepatotoxicity and cutaneous hypersensitivity that have been observed with this treatment.

Full more information and a full list of ESPT conferences, please visit www.esptnet.eu.

Financial & competing interests disclosure
The second ESPT conference couldn’t have been organized without the help of our sponsors, in particular
our gold sponsors (Bühlmann Laboratories, Randox and Roche), our silver sponsors (Agilent Technologies, Life Technologies, Affymetrix, Qiagen and Roche Diagnostics), our bronze sponsors (Luminex, Teva and Sequenom) and other support we obtained from DNA Genotek, Future Medicine, De Gruyter and Genomic Industry. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.
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The second European Society of Pharmacogenomics and Theranostics (ESPT) conference was organized in Lisbon, Portugal, and attracted 250 participants from 37 different countries. The participants could listen to 50 oral presentations, participate in five lunch symposia and were able to view 83 posters and an exhibition. Part 1 of this Conference Scene was presented in the previous issue of Pharmacogenomics. This second part will focus on: clinical implementation of pharmacogenomics tests; transporters and pharmacogenomics; stem cells and other new tools for pharmacogenomics and drug discovery; from system pharmacogenomics to personalized medicine; and, finally, we will discuss the Posters and Awards that were presented at the conference.

The meeting was organized by the European Society of Pharmacogenomics and Theranostics (ESPT) board members under the chairmanship of its president, with the local help of Carolino Monteiro, Sara Torgal, Luís Silva Santos and Pedro Gil, as well as the ESPT office in Nancy, France. Laurence Ben Rezig, the UMR INSERM U.1122 research team and Benjamin Richier for Com & Co, Marseille, France.

Clinical implementation of pharmacogenomics tests

Ron van Schaik (Erasmus University Medical Center, Rotterdam, The Netherlands) & Ramon Cacabelos (Camilo José Cela University, Madrid, Spain)

Ron van Schaik (Erasmus University Medical Center, Rotterdam, Netherlands) opened the session with a short overview on the challenges needed to be dealt with in getting pharmacogenetic markers into routine clinical care. Scientific evidence in itself just does not seem to be enough in a number of cases, he argued, although an increase in uptake of pharmacogenetic testing is currently visible. However, an exponential increase in publications on potentially relevant pharmacogenetic markers is also apparent, meaning there is an increased pressure to identify those markers fit for clinical implementation. At the Erasmus University Medical Center, TPMT, HLA-B*5701 and CYP2D6 are the most requested tests. When compared with other laboratories in the Netherlands, a survey by the Committee on Molecular Biological Testing of the Dutch Clinical Chemistry Association (NVKC) showed that CYP2D6 and CYP2C19 were the most frequently applied tests in routine diagnostics. This is due to the implementation of pharmacogenetic testing before initiation of therapy in several psychiatric clinics. When compared with, for instance, France, a completely different picture arises, with DPYD, IL28B, UGT1A1 and HLA-B*5701 being major tests. This indicates that huge differences exist not only between hospitals, but also between countries.

Marja-Liisa Dahl (Karolinska University Hospital, Stockholm, Sweden) shared her experiences of two decades of clinical pharmacogenetic testing at the Karolinska University Hospital. Most tests were based on predicting drug-metabolizing capacity and include CYP2D6, CYP2C19, CYP2C9, UGT1A1 and TPMT, and VKORC1 and HLA-B*5701 as non-metabolizing genes. Input from clinical pharmacology was indicated to be crucial for selecting the correct marker and variant alleles, as well as for interpretation. A need for national/international guidelines, standardized reports with clear information, evidence-based decision support, continued education of prescribers and systematic collection of data were put forward as important aspects to focus on.

Federico Innocenti (University of North Carolina, NC, USA) presented “Improving Cancer Drug Therapy Through the Patient Genome”. He discussed the UNC seq infrastructure set up at the University of North Carolina, which provides results to cancer patients based on sequencing their (cancer) genomes, and which is translated into actionable results.
Vangelis Manolopoulos (University of Thrace, Alexandroupulos, Greece), explained the predictive role of CYP2C9 and VKORC1 genotyping in coumarin treatment, and focused on the design of two prospective clinical trials on coumarin anticoagulant pharmacogenomics, for which the results are expected shortly.

Bruce Jordan (Roche Diagnostics, Rotkreuz, Switzerland) stepped in for Edith Schallmeier (Roche Diagnostics, Rotkreuz, Switzerland) with a presentation on “Future Outlook for Personalized Healthcare”. Showing the example of melanoma and BRAF mutation analysis, he underlined the power of companion diagnostics and demonstrated that diagnostic tests can significantly improve patient outcomes and enable a better utilization of healthcare resources. The areas of asthma, Alzheimer’s disease and schizophrenia were discussed in relation to companion diagnostics in the pipeline. The final message given by Jordan was that the growing number of targeted therapies in development will require innovative diagnostic tools to guide their use.

The next presentation was by Pedro Gil (Karolinska Institute and Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal) on “Pharmacogenetics and Treatment of Infectious Diseases in the Developing World”. With malaria as a proof of concept, Pedro showed the combination of pathogen and host genotyping by, preferably, a point-of-care machine.

Iris Grossman (TEVA Pharmaceutical Industries, Tel Aviv, Israel) finalized the session with a presentation on “Personalized Medicine & Pharmacogenomics (PMP) in drug R&D”. She indicated the formation of an R&D alliance between TEVA and Cancer Research technology (CNR) for cancer DNA damage response drugs. The idea is that the effect of genetic polymorphisms in modifying disease could be mimicked by drugs blocking that specific pathway, thus generating the ‘desired phenotype’. As an example, resistance to HIV infection among persistently seronegative prostitutes in Nairobi [1] eventually led to CCR5 monoclonal antibodies for HIV therapy. Another example was the development of a sodium channel blocker to treat Nav1.7-mediated pain in erythromelalgia.

Peter J Meier-Abt (University of Basel, Basel, Switzerland) & Carolino Monteiro (Universidade de Lisboa, Lisbon, Portugal)

Peter J Meier-Abt opened the session by giving an overview on the most important membrane transporter super families in vertebrate animal species including men. The human genome contains a total of 826 transport proteins encoding genes, among which 48% represent solute carrier (SLC) genes, 38% ion channels and ionotropic receptor genes, 6% ATP transporter genes and 8% other transport ion channels [2]. In particular, SLC and ATP-binding cassette (ABC) transporters have broad substrate specificities and exhibit a wide tissue distribution. They are involved in numerous physiological processes including neuronal transmission, intestinal organic substrate (including drugs) and iron absorption and the enterohepatic circulation of bile acids. Very interestingly, the hepatic sodium-dependent bile acid uptake system (NTCP) has been recently identified as the hepatitis B virus receptor.

Christoph Funk (F Hoffmann-La Roche Ltd, Basel, Switzerland) highlighted the importance of organic anion-transporting polypeptides (OATPs) and of P-glycoprotein (MDR1) for drug absorption, distribution and elimination. He especially emphasized the importance of a close interplay between OATP1B1-mediated drug uptake and subsequent cytochrome P450-dependent drug metabolism in the liver. The impact of major transporters on pharmacokinetic and safety profiles of potential new drug candidates must nowadays be studied early in the process of drug R&D.

Wolfgang Sadee (The Ohio State University, OH, USA) focused on the genomics and epigenomics of membrane transporters. New challenges are especially the numerous (‘pervasive’) interactions between the various classes of RNA such as ‘long and small noncoding RNAs’ and ‘protein encoding RNAs’. Specific mRNA transcripts encoding membrane transporters of human brain and liver can be identified by whole transcriptome sequencing. Furthermore, nicotine appears to influence
RNA–RNA interactions in specific brain regions. Also, regulatory polymorphisms affect not only gene transcription, but also RNA processing and translation, such as, for example, in CYP2D6 expression.

Matthias Schwab (Dr Margarete Fischer-Bosch-Institut für Klinische Pharmakologie [IKP], Stuttgart, Germany and University Hospital Tuebingen, Germany) emphasized the importance of DNA methylation in the expression of absorption, distribution, metabolism and excretion (ADME) genes, such as cytochrome P450 enzymes (e.g., CYP1A2), and of drug uptake (e.g., OCT1 in the liver; OCT2 in the kidney) and efflux transporters. Also, DNA methylation of hepatic SLC22A1 is associated with downregulation of SLC22A1 expression in hepatocellular carcinoma (HCC). However, like other epigenetic markers DNA methylation is also age-dependent, which might limit its general use as a biomarker for diagnosis and/or therapeutic response.

Ingolf Cascorbi (Institute of Experimental and Clinical Pharmacology, Kiel, Germany) started his presentation with an overview about the importance of microRNAs (miRs) and their impact on ABC transporter-mediated drug resistance. A variety of miRs were identified that interfere with ABCB1 (MDR1, P-glycoprotein), ABCC2 (i.e., miR-379) and ABCG2 (BCRP) expression. Interestingly, imatinib treatment was found to be associated with downregulation of miR-212 and miR-328 and overexpression of ABCG2, possibly contributing to the induction of imatinib resistance in chronic myeloid leukemia (CML). In general, the studies demonstrate that drug bioavailability is affected to a great extent by genetic–epigenetic interactions, supporting the concept that pharmacogenomics must be taken into account for better individualization of drug therapy.

Janja Marc (University of Ljubljana, Ljubljana, Slovenia) presented a study in 53 osteoporotic postmenopausal women investigating the role of the organic-anion transporting polypeptides OATP1B1 and OATP1B3 and their genetic variants in the pharmacokinetics and pharmacodynamics of raloxifene. The results indicate that SLC01B1 c.388A>G polymorphism could play an important role in the pharmacokinetics and pharmacodynamics of raloxifene.

Aurea Lima (Portuguese Institute of Oncology, Portugal) investigated the significance of the SLC19A1 G80A polymorphism for gastrointestinal toxicity of methotrexate (MTX) in 233 patients with rheumatoid arthritis. Interestingly, G carriers had a three- to seven-fold higher risk for gastrointestinal MTX toxicity as compared with AA homozygous patients, indicating that genotyping for the SLC19A1 G80A polymorphism might help to avoid gastrointestinal toxicity and to individualize MTX treatment in rheumatoid arthritis patients.

**Stem cells & other new tools for pharmacogenomics & drug discovery**

David Gurwitz (Tel-Aviv University, Tel Aviv, Israel) & João Queiroz (Rector University of Beira Interior, Covilha, Portugal) Michel Kranendonk (Universidade nova de Lisboa, Lisbon, Portugal) presented on in vitro tools for studying the pharmacogenetics of human cytochrome P450 enzymes, responsible for the phase I clearance of most drugs. The genes coding for these enzymes are highly polymorphic, and this polymorphism affects drug pharmacokinetic differences among individuals. He demonstrated the use of a new human P450 competent cell model for use in a high-throughput drug bioactivation assay, including an example of the study of drug-induced liver injury.

Lino Ferreira (University of Coimbra, Portugal) presented on the generation of an in vitro human blood–brain barrier (BBB) model. This BBB model is based on human cord blood-derived hematopoietic stem cells and is vital for CNS drug discovery and development for treating neurological and psychiatric diseases. The cord blood cells are initially differentiated into endothelial cells followed by the induction of BBB properties by their co-culture with pericytes. The brain-like endothelial cells were shown to express tight junctions and transporters typical for brain endothelium and to maintain expression of in vivo BBB properties for at least 20 days in vitro. This BBB model is reproducible, can be
generated from stem cells isolated from different donors with different CNS diseases, and utilized under flow conditions for toxicological assessment.

David Gurwitz presented on the utility of human lymphoblastoid cell lines (LCLs) cultures for discovering pharmacogenomic biomarkers. Such cell lines, hundreds of thousands of which are curated by numerous biobanks globally, comprise the most efficient method for representing and studying the natural human genome and epigenome variation (the human variome). The utility of this cell model was demonstrated by new findings from ongoing study on selective serotonin re-uptake inhibitors antidepressant response biomarkers.

Hans-Christoph Schneider (Sanofi-Aventis, Frankfurt, Germany) presented on modeling and simulation approaches for representing biochemical and physiological processes as dynamic mathematical models. Such models can be applied for running in silico simulations, saving huge costs by optimizing the choice of conditions of performing actual experiments during the strenuous drug development process (cohort choice and size, drug dosage, treatment periods etc.). This was demonstrated by a specific model applied for simulating a ‘virtual pancreatic β-cell’ for the development of two classes of antidiabetic drugs, the K-ATP-channel inhibitors (sulfonylureas) and the GLP1-receptor agonists. These in silico simulations reproduced all major aspects of these antidiabetic drugs as observed by in vivo corresponding experiments following the simulations.

Ramón Cacabelos presented on biomarkers for diagnosing and treating Alzheimer's disease (AD). Several studies suggest that genetics affect therapeutic outcome in AD; however, so far no genetic tests are used in the clinic for treating this disease. Genotypes for APOE, TOMM40 and CYP2D6 may explain some of the large variations in response of AD patients to therapeutic interventions. With increased prevalence reflecting population graying, and escalating societal costs, treating AD is becoming a major healthcare concern for developed countries. Decoding the pharmacogenomics of AD therapeutics will be instrumental for improving its treatment.

From system pharmacogenomics to personalized medicine

Gérard Siest (University of Lorraine, Nancy, France) & Helder Mota-Filipe (Infarmed, Lisbon, Portugal)

This was the last session, introduced by a speech from Gérard Siest on the importance of using a systems biology strategy for pharmacogenomics. Five sets of genes should be followed in a study of a drug with potential pharmacogenomic interaction: genes involved in pharmacokinetics – essentially, drug-metabolizing enzyme genes; genes involved in pharmacodynamics; genes involved in the pathologies to be treated; genes involved in the physiological variations, including environmental effects (tobacco, nutrition etc.); and, finally, additional genes involved in the biomarker variations used for the follow-up of the drug.

The strategy necessitates the use of the molecular definition of the disease and pathway approaches based on the metabolites, the proteins or peptides, and the mRNAs measured simultaneously with the DNA polymorphisms. The co-development of the drug with the biomarkers is also important.

We should work for and with the patient in the collection of all the necessary data, including the physiological measurements that could be done by specific devices on their body. Then, the P4 strategy will be followed: predictive, personalized, preventive and participatory in health and medicine.

Krishna Prasad (Saint Thomas Hospital, MHRA, London, UK), chair of the EMA committee of pharmacogenomics, presented some examples of the work carried out under EMA responsibility. The number of drugs examined was 116 during the last 8 years, and only 27 have relevant pharmacogenomics problems. Cytochromes were involved for five drugs and were discovered mainly after the drug had been put on the market or for generic drugs. For testing the efficacy, the number of patients is increasingly small reaching; as an example, there were only 100 patients for showing a benefit in gene therapy for LPL deficiency.

However, for safety testing it is more difficult. The quality of the biomarkers,
their cut-off points and the use of prospective studies are ideal, but with good biobanks, retrospective studies could also provide important results.

Helder Mota-Filipe, our host, is teaching pharmacology in the Faculty of Pharmacy, and he is co-director of the Portuguese Regulatory Organization. In examining the data on drug efficacy and safety, he was often able to give advice to the companies that are bringing files to the agency and explain the guidelines to them. He insisted on the biomarkers’ validation and their relevance to the different target organs of the drug, that is, the lung for gefitinib in the treatment of lung cancer.

The problem of the cost of treatments should not be neglected, and pharmacogenomics should help to reduce the cost of the treatment.

Howard J Federoff (Georgetown University Medical Center, Washington DC, USA) was developing a systems medicine approach for Parkinson’s disease, the second most common neurodegenerative disease with motor dysfunction and chronic neuroinflammation in the glia affecting the dopamine pathways. Using genome-wide association studies, exon sequencing and all the omics, current research in his laboratory is trying to understand Parkinson disease mechanisms and their genetics with a pathobiological model. In recent collaborative studies, Federoff and the network have shown the involvement of the complex mitochondrial respiratory chain with loss of dopamine neurons. Between the therapeutic targets, they found PPARα activator, reactive oxygen species pathways and SIRT1. Animal models of mice have been used to test the inflammatory pathways model with lipopolysaccharide administration that provoked neuroinflammation with decrease of dopamine neurons and PGC1 methylation. With these models many drugs have been tested, i.e., fenofibrate, which was able to protect cells with an anti-inflammatory affect. This safe and very well-tolerated drug should now be studied in humans on the basis of the previously described systems medicine approach.

Christina Kyriakopoulou (European Commission – DG Research and Innovation, Brussels, Belgium) guided the participants through the different funding opportunities at the European Commission health directorate. During the last years, personalized medicine linked to the different omics technologies was essentially willing to respond to several challenges for translating basic knowledge and applied research to the clinic. Cellular tools, biomarkers, molecular definition of diseases and patient stratification were some of the content of the last grant applications. 44 projects were supported on cellular signaling models and proof of concept in cancer or cardiometabolic and inflammatory diseases, multiple sclerosis and allergic rhinitis.

The vision for ‘Horizon 2020’ is a global strategy for all health-related projects. Three prerequisites for the grant applications are excellence, industrial leadership and citizen interest. There will be a strong focus on personalized health, including healthy aging.

Posters & awards
The second ESPT Conference ‘Pharmacogenomics: from cell to clinic’ organized in Lisbon in 2013 was an opportunity to gather and interact with young researchers in the field of pharmacogenomics and theranostics. In addition to the training course, students were invited to present their current work to a multidisciplinary poster committee: Professor Isabel Marques Carreira (Portugal), Dr Ndeye Coumba Ndiaye (France) and Professor Wolfgang Sadee (USA), under the chair of Professor Marja-Liisa Dahl (Sweden).

Eighty three posters from 23 countries were considered in Lisbon. After a first round, the jury preselected 21 outstanding posters that were further examined, and then delivered two ‘scientific awards’ at the end of the second round: the Federation for European Pharmacological Societies (EPHAR) [3] – ‘Pharmacogenomics Clinical Implementation’ Award was delivered to Dr Mi-Young Lee (Karolinska Institutet, Sweden) for her poster entitled ‘Reduced activity of cytochrome P450 2C9*35 allelic variant is determined by the altered interaction with P450 oxidoreductase’; and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) [4] – Pharmacogenomics Technical Tools Award was delivered to Dr Chiara Di Resta (Vita Salute San Raffaele University, Italy) for her poster...
entitled ‘Identification of new candidate genes in Brugada syndrome using the next generation sequencing’.

Dr Lee’s work is focused on cytochrome P450 2C9 (CYP2C9), one of the abundant CYPs, which hydroxylates many clinically important drugs including warfarin, losartan and diclofenac. Her team recently reported a new allelic variant, CYP2C9*35, which might be connected with the reduced activity of CYP2C9. The experimentations of Dr Lee et al., based on HEK293 cells, showed that the mutations in CYP2C9*35 abolish fruitful interactions with the P450 reductase, causing an inactive enzyme variant.

Dr Di Resta’s work applied recent advances in next-generation sequencing on a genetically heterogeneous pathology: Brugada syndrome (BS). 70% of this cardiac arrhythmic disorder’s patients remain genetically undiagnosed to date, so the project presented intended to increase the proportion of genetically diagnosed BS patients, identifying new BS-related genes and causative variants. In a lively presentation of her poster, Dr Di Resta highlighted the necessity to identify these variants in order to improve risk stratification and clinical management of asymptomatic patients.

The next ESPT conference will be the 7th Santorini Conference “Systems Medicine, Personalized Health and Therapy” – held September 25–27, 2014 in Santorini, Greece [5]. For more information and a full list of ESPT conferences, please visit [6].

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Financial & competing interests disclosure

The second ESPT conference couldn’t have been organized without the help of our sponsors, in particular our gold sponsors (Bühlmann Laboratories, Randox, Roche), our silver sponsors (Agilent Technologies, Life Technologies, Affymetrix, QIAGEN, Roche Diagnostics), our bronze sponsors (Luminex, Teva, Sequenom) and other supports we obtained from DNA Genotek, Future Medicine, De Gruyter and Genomic Industry. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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5. 7th Santorini Conference. www.santorini2014.org
6. ESPT conferences. www.esptnet.eu