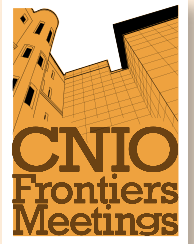




Genoma España



CNIO FRONTIERS Meetings 2010

Spanish National Cancer
Research Centre



Cancer Pharmacogenetics: personalizing medicine

Cancer Pharmacogenetics: personalizing medicine

22-24 **November** 2010
Madrid



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Javier Benítez, CNIO, Madrid, Spain

William E. Evans, St. Jude Children's Research Hospital, Memphis, USA

Magnus Ingelman-Sundberg, Karolinska Institutet, Stockholm, Sweden

Miguel Martín, Hospital Gregorio Marañón, Madrid, Spain



Cancer
Pharmacogenetics:
personalizing medicine

CNIO FRONTIERS Meetings 2010

CHAIRS' MESSAGE

Dear Colleagues,

It is our pleasure to welcome you to the first CNIO Frontiers Meeting about "Cancer Pharmacogenetics: individualizing medicine". This Conference aims to present recent advances in this field from a multidisciplinary point of view.

With the cost of sequencing rapidly approaching \$1,000 per human genome, the potential of personal genomic medicine is rapidly increasing. The identification of genome sequences that may be predictive of response to medication (Pharmacogenetics) is crucial for the progress of modern medicine. This new knowledge will be especially relevant for Oncology due to the narrow therapeutic indexes of most anticancer drugs and lack of efficacy. This advance will only be possible if researchers, industry, regulators, clinicians and policymakers work in cooperation. In this Meeting we intend to bring together top scientists in different fields of Pharmacogenetic research to discuss and plan future strategies to help translate this information into the cancer clinical care.

Thank you all for coming to Madrid and we hope you enjoy a pleasant and successful CNIO Frontiers Meeting.

Madrid, November 22-24th, 2010

Javier Benítez
William E. Evans
Magnus Ingelman-Sundberg
Miguel Martín



Cancer Pharmacogenetics: personalizing medicine

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Detailed Programme

MONDAY, NOVEMBER 22

9:15-9:30 **Welcome address**
Javier Benítez, CNIO, Madrid, Spain

Session 1: Inherited and somatic genetic variation and cancer pharmacotherapy

Chair: Rafael Rosell

- 9:30-10:05 **Magnus Ingelman-Sundberg**, Karolinska Institutet, Stockholm, Sweden
Pharmacogenetics and epigenetics of drug response
- 10:05-10:40 **Alex Sparreboom**, St. Jude Children's Research Hospital, Memphis, USA
Role of polymorphic transporters in taxane pharmacodynamics
- 10:40-11:15 **Julian Downward**, Cancer Research UK London Research Institute, London, UK
New approaches to targeting RAS signaling pathways in cancer
- 11:15-11:45 *Coffee break and poster session*
- 11:45-12:20 **Anna Di Rienzo**, The University of Chicago, Chicago, USA
Inter-individual and inter-ethnic variation in the transcriptional response to glucocorticoids
- 12:20-12:55 **Peter Campbell**, Wellcome Trust Sanger Institute, Cambridge, UK
Interrogating the architecture of cancer genomes
- 12:55-13:10 **Annabelle Ballesta**, INRIA, Le Chesnay, France
Short talk: *Towards personalization of the circadian delivery of Irinotecan: a combined mathematical and experimental study in mice*

13:10-13:25 **Ramón García Escudero**, CIEMAT, Madrid, Spain
Short talk: *Mouse p53-deficient carcinoma models are tools to predict human cancer outcome and treatment response*

13:30-15:00 *Lunch and poster session*

Session 2: Pharmacogenomic and epigenomic determinants of anti-cancer drug efficacy

Chair: Magnus Ingelman-Sundberg

- 15:00-15:35 **David A. Flockhart**, Indiana University School of Medicine, Indianapolis, USA
Clinical pharmacogenomics in the treatment of breast cancer
- 15:35-16:10 **Rafael Rosell**, Catalan Institute of Oncology, Barcelona, Spain
Personalizing targeted therapies in lung cancer
- 16:10-16:40 *Coffee break and poster session*
- 16:40-17:15 **Manel Esteller**, Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain
Pharmacoepigenetics and epigenetic drugs
- 17:15-17:30 **David Páez**, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
Short talk: *A genotype-directed phase I-IV study in advanced colorectal cancer treated with irinotecan in combination with fluorouracil/leucovorin: dose individualization*
- 17:30-17:45 **Virginia Pérez Andreu**, University of Murcia, Spain
Short talk: *Acenocoumarol pharmacogenetics and the path towards personalized medicine: An alternative modulation of VKORC1 expression by microRNAs*

TUESDAY, NOVEMBER 23

Session 3: Pharmacogenetics of cancer adverse effects

Chair: William E. Evans

- 9:30-10:05 **William E. Evans**, St. Jude Children's Research Hospital, Memphis, USA
Pharmacogenomics of acute lymphoblastic leukemia in children
- 10:05-10:40 **Howard McLeod**, University of North Carolina, Chapel Hill, USA
Pharmacogenetics of colon cancer therapy
- 10:40-11:15 **Anna González-Neira**, CNIO, Madrid, Spain
Pharmacogenetics in breast cancer therapy
- 11:15-11:45 *Group picture, coffee break and poster session*
- 11:45-12:20 **Cristina Rodríguez-Antona**, CNIO, Madrid, Spain
Identification of genetic variants associated to the adverse effects of microtubule-targeted anticancer drugs
- 12:20-12:35 **Susanna Leskelä**, Hospital Universitario de la Princesa, Madrid, Spain
Short talk: *miR-200 family controls β -tubulin III expression and is associated with paclitaxelbased treatment response and progression-free survival in ovarian cancer patients*
- 12:35-12:50 **Elixabet López**, University of the Basque Country, Leioa, Spain
Short talk: *Polymorphisms in SLCO1B1 gene: a new tool for MTX toxicity prevention*
- 13:00-14:30 *Lunch and poster session*

Sesion 4: State-of-the-art in high-throughput technologies and bioinformatic tools for pharmacogenetics

Chair: Peter Campbell

- 14:30-15:05 **Jörg D. Hoheisel**, German Cancer Research Centre (DKFZ), Heidelberg, Germany
Functional genomics and proteomics in cancer research
- 15:05-15:40 **Javier Benítez**, CNIO, Madrid, Spain
From pharmacogenetics to pharmacogenomics, the technological challengeology
- 15:40-16:15 **Arcadi Navarro**, Pompeu Fabra University, Barcelona, Spain
Genome-wide Association Studies Pipeline (GWASp): a desktop application for genome-wide SNP analysis and management
- 16:15-16:45 *Coffee break and poster session*
- 16:45-17:00 **Daniela Caronia**, CNIO, Madrid, Spain
Short talk: *Polymorphisms in ABCB1 and ABCC3 predict survival after treatment for osteosarcoma*
- 17:00-17:15 **Javier Leandro**, CNIO, Madrid, Spain
Short talk: *Identification of genetic markers of sunitinib efficacy and toxicity in first line treatment of renal clear cell carcinoma: a prospective multicenter study*

WEDNESDAY, NOVEMBER 24

Session 5: Implementing Pharmacogenetics: how industry, regulators, clinicians and policymakers are shaping the field

Chair: Miguel Martín

- 9:30-10:05 **Miguel Martin**, Hospital Gregorio Marañón, Madrid, Spain
Pharmacogenetics: implications for the design of cancer clinical trials
- 10:05-10:40 **Ron Van Schaik**, Erasmus University Medical Center, Rotterdam, The Netherlands
Barriers for pharmacogenetics implementation: a 6 year laboratory experience
- 10:40-11:15 **Christian Meisel**, Roche Pharma Research & Early Development, Penzberg, Germany
Fitting treatments to patients: implementing personalized healthcare in oncology R&D
- 11:15-11:45 *Coffee break and poster session*
- 11:45-12:20 **Bruno Flamion**, FUNDP-University of Namur, Belgium
A regulatory framework for pharmacogenomics-based drug development
- 12:20 **Closing remarks**
Magnus Ingelman-Sundberg, Karolinska Institutet, Stockholm, Sweden

Note: Talks: 25 minutes **Discussion:** 10 minutes after each talk

8 Short talks: 10 minutes each **Discussion:** 5 minutes after each short talk



Session 1

**Inherited and somatic
genetic variation and
cancer pharmacotherapy**

Chair: Rafael Rosell

Pharmacogenetics and epigenetics of drug response

Magnus Ingelman-Sundberg

Karolinska Institutet, Stockholm, Sweden

Pharmacogenomic research has focused on understanding the molecular mechanisms behind Adverse Drug Reactions (ADRs) and finding biomarkers that identify people at risk as well as finding genetic causes to altered efficacy of drugs. Serious ADRs have been shown to cause/contribute to 6-7% of all hospitalisations, a 2-day increase in average length of hospitalisation, 100,000 deaths annually in the US and, may according to some estimations have a similar cost as the drug treatment itself. During recent years the number of reported ADRs and ADR-related fatalities have actually increased, both by about 2.6-fold. At least 34 drugs were withdrawn from the market during 1995-2006, mainly due to hepatotoxic or cardiotoxic effects. The search for pharmacogenomic biomarkers has focused on variations in genes encoding drug-metabolizing enzymes. For immune-mediated toxicities, much focus has been placed on the MHC Class I genes. A review of pharmacogenomic biomarkers reveals that markers with significant specificity and sensitivity encompass variable genes encoding drug-metabolising enzymes and transporters in relation to treatment with some anticancer drugs (irinotecan, 6-mercaptopurines, irinotecan), codeine, antidepressants, clopidogrel, tamoxifen, simvastatin and warfarin. Highest specificity is seen amongst the HLA allelic variants where more specific interactions occur and where the ADRs caused by e.g. carbamazepine, flucloxacillin, ximelagatran and abacavir can be predicted at a relatively high specificity and sensitivity. It is plausible that further specific identification of HLA, HLB and HLC alleles can improve the ability to generally protect immune-mediated ADRs and eventually completely prevent such kind of ADRs in the future. However, this will be a complicated task in view of the several hundred thousands allelic variants in these loci.

The interindividual differences in drug metabolism are extensive. At present we do not understand a major part of the true genetic reasons to such variability as copy number variations, insertions/deletions and SNPs. Still the bases for a large extent of interindividual differences in enzyme expression or activity as revealed to be inherited from *in vivo* studies is extensive and remains to be elucidated. Such differences can be explained by epigenetic factors such as DNA methylation, posttranslational modification of histones and expression of ncRNAs such as microRNAs and RNAs. Among the P450s such regulation is to be expected to be of importance for e.g. the variation in CYP1A2 and CYP3A4 expression, where no functional genetic polymorphism has been found. In the lecture an update on the knowledge about epigenetic regulation of phase I and phase II will be given and future direction in this novel research field outlined. The lecture will also give an update in the field of genetic polymorphism of genes of importance for prediction of drug metabolism and ADRs focusing on clinically relevant examples.

Role of polymorphic transporters in taxane pharmacodynamics

Alex Sparreboom

St. Jude Children's Research Hospital, Memphis, USA

The taxanes paclitaxel and docetaxel are among the most widely used oncology drugs and have been approved for the treatment of breast, lung, ovarian, prostate, gastric, and head/neck cancers. The pharmacokinetic profiles of these agents are characterized by substantial interindividual variability, with up to 10-fold differences in drug clearance even in patients with normal hepatic function. Previously it was demonstrated that decreases in taxane clearance increase the odds of developing neutropenia, and that systemic exposure is a predictor of time to tumor progression in patients with NSCLC. The erratic or unpredictable response to paclitaxel and docetaxel remains a major challenge of modern chemotherapy. Recent population pharmacokinetic analyses have attempted to identify demographic and physiologic factors that may influence the clearance of these agents. However, the magnitude of interindividual pharmacokinetic variability still remains largely unexplained. In recent years, important new insights have been made relating to polymorphic liver transporters involved in paclitaxel and docetaxel elimination, such as the solute carriers OATP1B3 and OAT2, and the ABC transporters ABCB1 and ABCC2. Identification of genetic factors associated with interindividual variability in the disposition of taxanes is potentially vital to predicting or eventually adapting appropriate, individualized doses. However, traditionally, pharmacogenetic studies in oncology with these agents have been mostly retrospective, uncontrolled, and underpowered due to the limited number of patients evaluated that carry the variant genotypes of interest. In addition, genotype-phenotype association studies have typically focused on single candidate genes, or even single variants without consideration of the multiple-gene contributions and the complexity of the disposition characteristics of these agents. Furthermore, the possible clinical impact of inherited genetic variation in taxane-containing chemotherapeutic regimens may be dependent on disease type, drug dose, schedule, and/or concurrent combination therapy. Integrated approaches combining genetic and environmental information in large-scale population studies is required to further advance this field.

New approaches to targeting RAS signaling pathways in cancer

Julian Downward

Cancer Research UK London Research Institute, London, UK

The RAS oncogene is very frequently activated in human tumours and, as a result, the signaling pathways it controls, such as RAF/ERK and PI3-kinase/Akt, have been well studied. However, effective targeting of these pathways as a therapeutic approach to cancer has remained elusive. In order to find novel targets in RAS oncogene signaling pathways, we have undertaken a number of studies using large-scale RNA interference libraries. One has been a screen for genes that cause apoptosis in RAS oncogene addicted cells.

In this way a number of pathways have been identified that are important for survival of RAS transformed, but not normal, cells. Some of these have not previously been implicated in RAS signaling. Targeting synthetic lethality and oncogene addiction may provide optimal differential killing of RAS mutant cancer cells relative to normal cells.

One of the biggest problems in cancer therapy is the development of resistance to treatments that may be initially quite effective. The enormous selective pressure imposed by the therapy on the genetically unstable tumour can lead to rapid emergence of resistant clones of cells. We have investigated the mechanisms by which resistance can arise. In the case non-small cell lung cancer with activating EGF receptor mutations (about 10% of total cases), EGF receptor tyrosine kinase inhibitors can be very effective initially. However, resistance can be caused by second site mutations in EGFR (such as T790M), or by other mechanisms such as MET amplification. We have identified a number of target genes whose loss of function can result in resistance to these drugs, and provide evidence that at least one of these occurs in tumours, resulting in activation of drug-independent signaling driven by RAS downstream of the EGF receptor.

Inter-individual and inter-ethnic variation in the transcriptional response to glucocorticoids

Anna Di Rienzo

The University of Chicago, Chicago, USA

Glucocorticoids (GCs) are widely used as cotreatment for a variety of cancers because of their potent proapoptotic properties and because they provide symptomatic relief. Extensive inter-individual and inter-ethnic variation has been observed in the clinical response to GCs. While some non-genomic GC effects have been observed, GCs are thought to act primarily through the regulation of target gene transcription. Therefore, regulatory polymorphisms that impact the transcriptional response to GCs may contribute substantially to inter-individual and inter-ethnic variation in treatment response and side effects. We have used an in vitro cell line system to characterize the transcriptional response to GC treatment at the genome-wide level in populations of European and African ancestry. We find substantial inter-individual and inter-ethnic variation in response. This variation is in large part attributable to genetic factors, some of which vary in frequency across ethnic groups. While the majority of these factors affect expression levels of GC target genes in a treatment-independent manner, a minority shows significant effects only in the presence or, respectively, the absence of treatment. These genetic variants are strong candidates for predicting response to GC treatment.

Interrogating the architecture of cancer genomes

Peter Campbell

Wellcome Trust Sanger Institute, Cambridge, UK

Cancer is driven by mutation. Using massively parallel sequencing technology, we can now sequence the entire genome of cancer samples, allowing the generation of comprehensive catalogues of somatic mutations of all classes. Bespoke algorithms have been developed to identify somatically acquired point mutations, copy number changes and genomic rearrangements, which require extensive validation by confirmatory testing. The findings from our first handful of genomes illustrate the potential for next-generation sequencing to provide unprecedented insights into mutational processes, cellular repair pathways and gene networks associated with cancer development. I will also review possible applications of these technologies in a diagnostic and clinical setting, and the potential routes for translation.

Towards personalization of the circadian delivery of Irinotecan: a combined mathematical and experimental study in mice

Annabelle Ballesta^{1, 2}, C. Ahowesso², S. Dulong², E. Piccolo³, X. Li², J. Clairambault^{1, 2} and F. Levi²

¹INRIA, BANG project, Domaine de Voluceau BP.105, Le Chesnay, France; ²INSERM, U776 Rythmes Biologiques et Cancers, Hôpital Paul Brousse, Villejuif, France; ³CINBO (Consorzio Interuniversitario Nazionale per la Bio-Oncologia), Chieti, Italy

Irinotecan (CPT-11) is an anticancer drug currently in clinical use for the treatment of colorectal cancer. Its efficacy and toxicity are largely influenced by the time of the day (or circadian time) it is administered to both in mice and in patients. Recent findings highlighted the fact that optimal circadian delivery may highly depend on the patient gender and genetic background ([1]). An experimental study in mice allowed the determination of three classes which displayed different rhythms of Irinotecan chronotoxicity. Namely class1 (B6D2F1, female), class 2 (B6D2F1, male) and class 3 (B6CBAF1, female) displayed best CPT11 tolerability at respective time of injection ZT15, ZT11 and ZT15, and worst tolerability at ZT3, ZT23 and ZT7 (ZT= Zeideberg Time, ZT 0 being light onset).

Irinotecan pharmacokinetics (PK- what the cells do to the drug, e.g. metabolism, transport), and pharmacodynamics (PD- what the drug does to the cells, e.g. DNA damage) are largely influenced by 24-hour-period rhythms of certain proteins including the drug target Topoisomerase I, the activation enzymes (Carboxylesterases), the deactivation enzymes (UGT1A1, UGT1A9) and the ABC transporters which are responsible for the drug efflux. In order to identify molecular biomarkers which could discriminate between the classes and to design optimal circadian delivery pattern for each of them, a whole body physiologicallybased PK-PD model was built starting from a previous mathematical model designed thanks to a cell culture study ([2]). Parameters were estimated for each mouse class by fitting available data on tissular PK for two different circadian times of administration and on circadian rhythms of relevant proteins. Validation of the mathematical model by comparing its output with other experimental data is in progress. Then the parameter sets will be compared in order to find molecular differences between the classes and optimization algorithms will be applied to the model to design theoretically optimal chronomodulated scheme of administration. This study in mice may give a hint for determining molecular biomarkers which should be measured in patients in order to design a tailored optimal scheme for Irinotecan circadian delivery.

1. F. Lévi, A. Okyar, S. Dulong, Innominato PF., J. Clairambault, Circadian timing in cancer treatments, *Annu Rev Pharmacol Toxicol.* 2010;50:377-421. 2. A. Ballesta, S. Dulong, C. Abbara, B. Cohen, A. Okyar, J. Clairambault, F. Levi. A Combined Experimental and Mathematical Approach for Molecular-based Optimization of Irinotecan Circadian Delivery. Article in preparation and A. Ballesta's PhD thesis, 2010.

Mouse p53-deficient carcinoma models are tools to predict human cancer outcome and treatment response

Ramón García-Escudero, Ana B. Martínez-Cruz, Mirentxu Santos, Concha Bielza, Pedro Larrañaga and Jesús M. Paramio

Molecular Oncology Unit, Division of Biomedicine, CIEMAT, Madrid, Spain; Departamento de Inteligencia Artificial, Universidad Politécnica de Madrid, Campus de Montegancedo, Boadilla del Monte, Madrid, Spain

The epidermal specific ablation of p53 gene leads to the spontaneous development of invasive primary epidermal tumors in mice through a process that is accelerated by the simultaneous ablation of the retinoblastoma protein gene. Using crossspecies comparison and meta-analytical approaches of gene expression profiling, we have previously showed that mouse tumors display robust similarities with human tumors bearing mutated TP53, or displaying poor prognostic outcome, from multiple body tissues. Based on these analyses, we have described biomarkers that distinguish patients of different clinical outcome from three cancer types: breast cancer, multiple myeloma and astrocytoma. Combining our epidermal mouse tumors with tissue-specific human cancer gene expression profiling using a new method, we have obtained a genomic predictor of clinical outcome for breast cancer. The predictor calculates a risk score for each patient based on expression levels of 40 Affymetrix probesets corresponding to 31 genes. The method has been successfully validated in 12 published external datasets, representing more than 2300 individual primary breast tumors, performed in either Agilent or Affymetrix platforms (U133A, U133 Plus 2.0, or U133AAofAv2 Genechips), and analyzing different clinical endpoints such as loco-regional and/or distant recurrence, or death. In multivariate Cox regression analysis, 40-gene distinguishes risk patient groups independently of current clinical parameters (tumor size, tumor grade, ER status and patient age) and lymph node involvement. Furthermore, the genomic method also predicts outcome in tamoxifen-treated patients, and pathological complete response of patients treated with neoadjuvant paclitaxel plus fluorouracil, adriamycin and cyclophosphamide chemotherapy. The analyses demonstrate that p53-deficient mouse models are excellent tools to develop genomic predictors for clinical outcome of human cancer.

NOTES _____



Session 2

**Pharmacogenomic and
epigenomic determinants
of anti-cancer drug
efficacy**

Chair: Magnus Ingelman-Sundberg

Personalizing targeted therapies in lung cancer

Rafael Rosell

Catalan Institute of Oncology, Barcelona, Spain

Non-small-cell lung cancer (NSCLC) driven by EGFR mutations occurs predominantly in never-smokers, women and non-squamous cell histologies. In the European population, the overall frequency of EGFR mutations is 15-17%; however, this can reach 40% in never-smokers, compared to 10% in former smokers and 5% in current smokers. In females, the probability of finding mutations is 30%, compared to 10% in males. The predominant histological subtype of adenocarcinoma could have some influence on the frequency of EGFR mutations although more clinical data is needed. A large prospective study in patients with EGFR mutations showed that response to erlotinib was 70%, progression free survival was 14 months, and median survival was 27 months. Acquired resistance has been related to the presence of the EGFR T790M mutation, and specific inhibitors of the T790M mutation are promising. Additionally, the overexpression of MET has been reported to be relevant. The detection of T790M is a technical issue that can be solved with an adequate laboratory assay. We have detected EGFR double mutations (T790M plus deletions or L858R) in 35% of pretreatment biopsies from 129 NSCLC patients and observed a negative correlation with progression free survival to erlotinib. The baseline gene expression of other receptor tyrosine kinases, such as MET, AXL, IGF-1R and IL-6 did not influence erlotinib outcomes.

Erlotinib can cause double-strand breaks that are repaired mainly by homologous recombination. In experimental models, erlotinib sensitivity is highly influenced by BRCA1 status. In our experience, low levels of BRCA1 mRNA can prolong progression free survival to 27 months. In the multivariate analysis of progression free survival, only T790M, BRCA1 and PP2A/C were independent prognostic markers. In the multivariate analysis of survival, only T790M and BRCA1 were identified as independent markers.

Based on our findings, we do not believe that T790M is the main cause of acquired resistance. Rather, we speculate that the amplification of the pathway of H2AX/RNF8/RNF168/RAP80/BRCA1 could be a main cause of resistance to protracted treatment with erlotinib. At present, we are investigating the role of BRCA1 SUMOylation and sensitivity to erlotinib.

Pharmacoeugenetics and epigenetic drugs

Manel Esteller

Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain

The most promising epigenetic candidates to predict pharmacoeugenetic response are the DNA repair genes undergoing epigenetic inactivation in tumors, such as the O⁶-methylguanine DNA methyltransferase (*MGMT*), the mismatch repair gene *hMLH1*, the Werner gene (*WRN*) or the breast cancer susceptibility gene *BRCA1*. In healthy tissues these enzymes are responsible for repairing the DNA damage that occurs during our lifetime and prevent the formation of mutations and other type of genomic damage. However, in cancer cells they become our terrible foes because they repair the DNA damage induced by many used chemotherapy agents, thus, generating chemoresistance to drugs. However, there is another side to this story: these DNA repair genes undergo hypermethylation-associated silencing in a fraction of human tumors that progress with a mutator pathway phenotype, but this is also an Achilles' heel because these hypermethylated-malignancies will not be able to repair the DNA damage caused by the chemotherapy agent. With the emerging epigenomic technologies we now have the techniques that will help address the DNA methylation chemoprofiles in an unbiased manner, and complete the promising pharmacoeugenetics landscape. The recent unmasking of genetics lesions in tumors has optimized cancer treatment regimens, specifically with the identification of the presence of c-ERBB2/neu oncogene amplifications, the BCR-ABL translocations and EGFR mutations. It is predicted that in coming years, the hypermethylation patterns of particular genes will also predict response to specific treatments.

A genotype-directed phase I-IV study in advanced colorectal cancer treated with irinotecan in combination with fluorouracil leucovorin: dose individualization

David Páez¹, Laia Paré², Juliana Salazar^{2,3}, Elisabeth del Rio², Agustí Barnadas¹, Eugenio Marcuello¹ and Montserrat Baiget²

¹Medical Oncology and ²Genetics Departments, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; ³U-705 CIBERER, Barcelona, Spain

Infusional fluorouracil/Leucovorin (FU/LV) plus irinotecan (FOLFIRI) is one of the standard first-line options for patients with metastatic colorectal cancer (mCRC). Irinotecan is converted into 7-ethyl-10-hydroxycamptothecin (SN-38) by a carboxylesterase and metabolised through uridine diphosphate glucuronosyl transferase (UGT1A1). The UGT1A1*28 allele has been associated with the risk of developing severe toxicities.

The present trial was designed to define the maximum tolerated dose according to UGT1A1 genotype. This report focuses on the results of tolerance to different escalated doses of FOLFIRI first-line of chemotherapy. Patients undergoing first-line treatment for mCRC eligible for treatment with FOLFIRI were classified according to UGT1A1 genotype. Eighty-eight patients were eligible for dose escalation of irinotecan. The starting dose of biweekly irinotecan was 180mg/m² for the *1/*1, 110 mg/m² for the *1/*28 and 90mg/m² for the *28/*28 genotypes.

The dose of irinotecan was escalated to 450mg/m² in patients with the *1/*1 genotype and to 390mg/m² in those with the *1/*28 genotype. Neutropenia and diarrhea were the most common grade 3 or 4 toxicities. Our results concluded that the recommended dose of 180mg/m² for irinotecan in FOLFIRI is considerably lower than the dose that can be tolerated for patients with the UGT1A1 *1/*1 and *1/*28.

Acenocoumarol pharmacogenetics and the path towards personalized medicine: An alternative modulation of VKORC1 expression by microRNAs

Virginia Pérez Andreu, R. Teruel, R. González Conejero, J. Corral, V. Roldán, N. García Barberá, V. Vicente and C. Martínez

University of Murcia, Centro de Hemodonación, Murcia, Spain; Hospital Morales Meseguer, Department of Hematology and Oncology, Murcia, Spain

One current focus of pharmacogenetics is the incorporation of miRNAs as a way of controlling protein expression with potential consequences on drug response. Objective: To determine the influence of miRNAs on VKORC1 expression and to evaluate the potential effect of one polymorphism located at the 3'UTR of VKORC1 mRNA on miRNA interaction.

In silico studies identified three putative binding sites for miR-133a, miR-137, and miR-147b on VKORC1 mRNA. These three miRNAs were expressed in healthy human liver. Transfection of miRNA precursors in HepG2 cells reduced VKORC1 mRNA expression in a dose-dependent manner as assessed by qRT-PCR. Luciferase reporter assays in HEK293T cells showed that all three miRNAs could interact with the 3'UTR of VKORC1 by reducing luciferase expression, whereas no effect on luciferase activity was observed in 3'UTR mutated for the respective miRNA binding-sites. Additionally, co-transfection of miRNA precursors in HEK293T cells, with a modified c-myc-tagged VKORC1 expression vector containing 3'UTR, confirmed a direct interaction of miR-133a and miR-147b with VKORC1 mRNA. MiR-137 might have an indirect effect on VKORC1 expression, as measured by western blotting and densitometric assays. The VKORC1 3730G>A (rs7294) polymorphism, located at the miR-147b binding site and near the miR-133a one, interfered with miR-147b and miR-133a binding, as measured by densitometric assays using a VKORC1 expression vector. A retrospective study in 4000 patients under anticoagulant therapy showed a modest but functional effect of rs7294, independently from rs99232321, on acenocoumarol dose requirements.

MiR-133a, miR-137, and miR-147b exert a regulatory effect on VKORC1 expression. A large analysis including almost four thousand patients under acenocoumarol therapy, confirms the role of rs7294 on acenocoumarol dose requirements. Our results strongly suggest that the functional effect of rs7294 on anticoagulant dose requirement



Session 3

**Pharmacogenetics of
cancer adverse effects**

Chair: William E. Evans

Pharmacogenomics of acute lymphoblastic leukemia in children

William E. Evans

St. Jude Children's Research Hospital, Memphis, USA

Pharmacogenomics is playing an increasingly important role in optimizing the treatment of many human diseases, including the treatment of childhood acute lymphoblastic leukemia (ALL). This presentation will focus on pharmacogenomic determinants of toxicity to ALL chemotherapy, and will not comprehensively address the growing body of data indicating that pharmacogenomics can provide insights into ALL treatment efficacy and drug resistance. Examples to be addressed in this presentation include three of the most common drug-induced toxicities from ALL chemotherapy; bone marrow toxicity (e.g. *TPMT*, *ITPA*), gastrointestinal toxicity (*SLCO1B1*) and osteonecrosis (*ACP1*, *SH3YL1*). There will also be brief discussion of pharmacogenomic determinants of the worst adverse drug effect in ALL treatment, poor efficacy. Advances in childhood ALL have long served as a model for curing disseminated human cancers, and now serve as a paradigm for exploiting pharmacogenomics to minimize toxicities and enhance efficacy of cancer chemotherapy. Background information can be found in Relling et al, *Lancet Oncol*, 2010; Trevino et al, *Nature Genetics* 2009; Pui et al, *NEJM*, 2009; Cheek and Evans, *Nature Rev Cancer*, 2006; Evans and Relling, *Nature*, 2004; Holleman et al, *NEJM*, 2004; Evans and McLeod, *NEJM*, 2003.

Pharmacogenetics of colon cancer therapy

Howard McLeod

University of North Carolina, Chapel Hill, USA

The field of pharmacogenomics has seen some exciting advances in the recent past. The Human Genome Project and International HapMap projects have uncovered a wealth of information for researchers. This has led to the discovery of clinically predictive germline genotypes (e.g. UGT1A1*28-irinotecan, TYMS TSER-fluoropyrimidines, CYP2D6-tamoxifen), germline haplotypes (e.g. VKORC1 Haplotype A-warfarin) and somatic mutations (e.g. epidermal growth factor receptor-gefitinib/erlotinib, KRas-cetuximab/panitumumab). The introduction of FDA approved pharmacogenetic tests (UGT1A1*28) and the initiation of a genotype-guided clinical trial for cancer therapy (TYMS TSER in rectal cancer) have provided the first steps towards the integration of pharmacogenomics into clinical practice. It is also clear that there are many barriers to clinical application. These include expanding the science to understanding the pathways of genes that regulate a drug's activity. There are also critical non-science issues, such as integration of new tests into health systems, changing old habits to allow application of new data, and the reality that the cost of both testing and the therapeutic options are a key driver in health care. As the scientific evidence matures, we must think beyond our favorite aspect of translational science if we are to overcome the many obstacles to delivering more careful selection of cancer therapy.

Pharmacogenetics in breast cancer therapy

Anna González-Neira

CNIO, Madrid, Spain

Although the current breast cancer therapies are improving rapidly, it is often hindered by drug resistance and treatment-related toxicities. Interindividual and interethnic variability in drug pharmacokinetics and pharmacodynamics may be explained by commonly occurring genetic polymorphisms in drug-metabolizing enzymes and drug transporters. Although to date pharmacogenetics studies have been focused on a reduced number of genes, the development of the new technologies are allowing researchers to focus these studies on the integration of multiple drug pathways and also to perform analysis at the genome-wide level. These new approaches allow a more comprehensive analysis of genetic factors influencing drug efficacy and toxicity in breast cancer and could reveal possible unknown mechanisms involved in drug response.

Identification of genetic variants associated to the adverse effects of microtubule-targeted anticancer drugs

Cristina Rodríguez-Antona

CNIO, Madrid, Spain

Microtubules are ubiquitous polymers critically involved in various cellular functions such as mitosis, intracellular transport and maintenance of the asymmetric morphology of cells. Several anticancer drugs extensively used and effective in a wide range of tumor types base their activity on the alteration of microtubule dynamics (e.g. taxanes and Vinca alkaloids). These drugs bind the tubulin subunits and arrest cells in mitosis finally causing cell death. The most relevant adverse effects of these drugs, hematologic toxicity and neurotoxicity, are among the major obstacles to improve the survival and quality of life of many cancer patients and lead to dose reductions, treatment suspensions and irreversible damages in some patients. However, the individual risk of each patient to develop severe toxicities exhibits a large inter-individual variability that cannot be predicted and, currently, drug treatment is not individualized. Therefore, efforts to discover biomarkers predictive of therapy outcome for these drugs are critical.

Genetic variation has been shown to have a large impact on the effects of many drugs. Thus, we aimed to identify polymorphisms associated with increased risk of drug adverse effects in microtubule-targeted anticancer drugs to improve therapy through an individualization and rationalization of the treatments based on genetic traits. To address this challenge through a full genomic perspective, we are using candidate gene approaches and also incorporating the recent developments of high-throughput technologies.

Using a candidate gene approach focused on genes involved in paclitaxel pharmacokinetics, we have identified three common functional polymorphisms altering cytochrome P450 activity associated to paclitaxel neurotoxicity (HR=1.64; per paclitaxel-metabolism-increasing allele, P=0.0003). Concerning candidate gene approaches for pharmacodynamics, we have focused on the most frequent therapeutic target of the microtubule-targeted anticancer drugs, the beta-tubulin. We have characterized the expression and genetic variation of the eight different human beta-tubulin isotypes and by using in vitro functional assays, we have identified polymorphisms altering taxanes action. In addition, genome-wide approaches are being conducted with samples from patients treated with paclitaxel.

miR-200 family controls β -tubulin III expression and is associated with paclitaxelbased treatment response and progression-free survival in ovarian cancer patients

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Ovarian cancer remains one of the leading causes of cancer deaths. Thus, new biomarkers predictive of response to the standard paclitaxel-carboplatin treatment are needed to improve chemotherapy strategies. MicroRNAs have the potential to modify drug outcomes. Based upon this, here we demonstrate that patients with high miR-200 family expression show low levels of β -tubulin class III in ovarian carcinoma. In addition, we establish the clinical relevance of these microRNAs for ovarian cancer patients' treatment response and survival. In a well characterized series of 72 ovarian carcinomas, the expression of miR-141, miR-200a, miR-200b, miR-200c and miR-429 were quantified by qRT-PCR and the protein content of β -tubulin isotypes I, II and III were determined by immunohistochemistry. The relationship between these microRNAs, β -tubulin expression, response to paclitaxel-based treatment, progression-free and overall survival, was determined. While isotype I had constant high levels, protein expression of β -tubulin II and III was mutually exclusive. Low tumoral miR-200 expression was significantly associated with high β -tubulin III protein content (P values range, 0.047 to <0.0001) and patients without complete response had lower miR-200c levels than patients with complete response (HR=1.43, 95%CI=1.02-1.99, P=0.037, multivariate analysis). Additionally, low miR-200 family expression had a trend towards poor progression-free survival (HR>2.0, P values 0.051, 0.054 and 0.079, for miR-200c, miR-141 and miR-429, respectively, multivariate analysis). In conclusion, miR-200 family members affect the final β -tubulin III protein content of ovarian carcinomas. Furthermore, these microRNAs might constitute biomarkers of response to paclitaxel-based treatments and relapse/progression of advanced stage ovarian carcinoma patients.

Polymorphisms in SLCO1B1 gene: a new tool for MTX toxicity prevention

Elixabet López López², A. Navajas¹, I. Martín Guerrero², B. Santos³, J. Uriz⁴, N. García de Andoin⁴, P. García Miguel⁵, M. A. Pinan⁶ and A. García Orad²

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Methotrexate (MTX) is an important component of the therapy in childhood acute lymphoblastic leukemia. Treatment with high-dose (MTX) often causes toxicity, dose reduction or cessation of treatment being necessary. Polymorphisms in genes involved in the (MTX) metabolism have been associated with toxicity with controversial results, possibly due to different factors, such as non-homogeneous toxicity criteria, population or treatment.

The aim of the present study was to analyze the implication of polymorphisms in genes involved in the methotrexate metabolism (MTHFR, TS, SHMT1, RFC1, ABCB1, ABCG2 and SLCO1B1) upon its toxicity during therapy with the well established LAL/SHOP protocol. This study was performed in 115 Spanish pediatric B-ALL patients, using methotrexate plasma concentration as objectively quantifiable toxicity criteria.

We confirmed the suitability of methotrexate plasma levels as toxicity criteria. We found a statistically significant association between methotrexate plasma concentration and rs11045879 CC genotype in SLCO1B1 (72 h, p=0.030; 96 h, p=0.011), supporting the results published in a recent work by Treviño and collaborators. We did not find any significant association in the other genetic polymorphisms analyzed, contradicting previously described associations.

Polymorphisms in the SLCO1B1 gene could be a useful tool for monitoring MTX-related toxicity in childhood Acute Lymphoblastic Leukemia.



Session 4

State-of-the-art in high-throughput technologies and bioinformatic tools for pharmacogenetics

Chair: Peter Campbell

Functional genomics and proteomics in cancer research

Jörg D. Hoheisel

German Cancer Research Center (DKFZ), Heidelberg, Germany

Our research aims at the analysis, assessment and description of both the realisation and regulation of cellular functions from genetic information. To this end, also new technologies are being developed for immediate application in research and – in longer terms – clinical routine.

Analyses on tumour material are at the centre of attention with an emphasis on pancreatic cancer. Parallel studies at a global level are under way on the epigenetic modulation of gene promoters, variations in transcription factor binding, changes of transcript levels of coding and non-coding RNAs, on the actual protein expression and the intensity of protein interactions. From the resulting data, we aim at an understanding of cellular regulation and its biological consequences.

In combination with clinical facts, the knowledge is used for the creation of means of early diagnosis, patient stratification, accurate prognosis, monitoring of treatment results as well as the establishment of new therapeutic avenues. For the last, several compounds are being investigated in combination with efficient, genome-wide knock-down assays and based on screens for druggable targets.

A more recent line of work aims at an *in vitro* implementation of complex biological processes. Motivation is a utilisation for the production of molecules and the establishment of artificial molecular systems. Cell-free biosynthetic production will become important for many biotechnological and pharmacochemical challenges ahead.

From pharmacogenetics to pharmacogenomics, the technological challenge

Javier Benítez

CNIO, Madrid, Spain

Environmental factors such as diet, age, gender or lifestyle can influence a patient's response to therapeutic treatments. However genetic factors are believed to be the key to maximize drug efficacy and minimize adverse side effects. Oncology is one of the most exciting fields because of the severity of adverse events and the high likelihood of mortality associated with nonresponse. Pharmacogenetics and pharmacogenomics hold the promise that therapeutic decision would be based on each individual patient's own genetic makeup.

Previous studies on some well characterized candidate genes (e.g drug metabolizing enzymes) have suggested that DNA variations (SNPs) can influence their ability to convert and efficiently eliminate drugs from the body. Some examples include variants in the TPMT gene and 6-mercaptopurine toxicity; UGT1A1 polymorphisms and irinotecan toxicity, or cytochrome P4502D6 variants and failure in the tamoxifen treatment. However the development of the Human Genome Project and highthroughput genotyping platforms permit currently the study of several genes from specific pathways or regions, or even from the whole genome (GWAS). This type of studies doesn't require a priori assumptions and therefore, applied unbiased approach. This pharmacogenomic –based strategy has been applied to identify candidate loci associated with response to treatment for various diseases although replication of these GWAS is a challenge because clinical trials based on a GWAS are from much collaboration which are unique per se. On the other hand GWAS have been focused on characterizing common genetic variants with allele frequencies greater than 5% and then, rare SNPs with large effects cannot be identified. In this way, next generation sequencing technologies can be very important in the identification of novel SNPs no previously identified by GWAS and in fact, the first pharmacogene database of 39 pharmacogenetic candidate genes from the 1000 genome project has just being released including a substantial number of novel SNPs.

Genome-wide Association Studies Pipeline (GWASpi): a desktop application for genome-wide SNP analysis and management

Arcadi Navarro

Pompeu Fabra University, Barcelona, Spain

Genome-wide Association Studies (GWAS) based on Single Nucleotide Polymorphism (SNP) arrays are the most widely used approach to detect loci associated to human traits. Due to the complexity of the methods and software packages available, each with its particular format requiring intricate management work-flows, the analysis of GWAS usually confronts scientists with steep learning curves. Indeed, the wide variety of tools makes the parsing and manipulation of data the most time-consuming and error prone part of a study. To help solving these issues, we present GWASpi, a user-friendly, multi-platform, desktop-able application for the management and analysis of GWAS data, with state of the art database technologies to extract the most out of commonly available desktop hardware. GWASpi is a "start to end" GWAS management application, from raw data to results, containing the most common analysis tools. Not only is GWASpi easy to use, but it reduces in up to two orders of magnitude the time needed to perform the fundamental steps of a GWAS.

Polymorphisms in ABCB1 and ABCC3 predict survival after treatment for osteosarcoma

Daniela Caronia¹, A. Patiño-García², G. Pita¹, L.T. Moreno¹, A. Pérez Martínez³, M. Zalacaín Díez², B. Molina³, I. Colmenero³, MR. Alonso¹, L. Sierrasesumaga Ariznabarreta², J. Benítez^{1,4} and A. González Neira¹

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Standard treatment for osteosarcoma involves neoadjuvant therapy with a combination of cisplatin, adriamycin and methotrexate before surgical resection of the primary tumour, followed by postoperative chemotherapy. However, many patients relapse after treatment or develop severe adverse events. In this study we assessed the impact of single nucleotide polymorphisms (SNPs) and also copy number variants (CNVs) in the chemotherapeutic metabolic pathway and transporter genes on clinical response and toxicities in osteosarcoma patients. We genotyped 366 SNPs using Veracode technology and 2 CNVs using Taqman assays in 24 genes involved in the drug metabolism and influx/efflux of cisplatin, adriamycin, methotrexate, vincristine, cyclophosphamide. We studied the association of the genotypes with tumour response, overall survival, and treatment-related adverse events in 91 osteosarcoma patients. We found 3 SNPs in two ATP-binding cassette genes significantly associated with overall survival: one SNP was located in the ABCC3 gene (HR=8.14, pvalue= 5.1x10⁻⁵), and two in the ABCB1 gene (HR of respectively 3.66 and 0.24, pvalue of respectively 6.9x10⁻⁵ and 7.9x10⁻⁵). Association of these SNPs remained significant after Bonferroni correction. In conclusion, in the present study we have identified new candidate genetic markers that, after an independent validation, could improve the treatment for osteosarcoma patients, helping in the design of individualized therapy.



Session 5

**Implementing
Pharmacogenetics:
how industry, regulators,
clinicians and policymakers
are shaping the field**

Chair: Miguel Martín

Barriers for pharmacogenetics implementation: a 6 year laboratory experience

Ron Van Schaik

Erasmus University Medical Center, Rotterdam, The Netherlands

The fact that genetic variants for drug metabolizing enzymes explain variation in activity between individuals dates from the 1990s. Since 2000, publications on pharmacogenetics have increased exponentially, and now reaches over 900 papers per year. New genetic variants and new associations are published. With all information available, it is surprising how slow integration of pharmacogenetics into routine diagnostics is going. Molecular biology knowledge expanded the last 20 years, genotyping techniques improved, costs declined and genetic tests became available more widespread, but only recently there seems to be some uptake of pharmacogenetics for diagnostics.

At our department, pharmacogenetic testing is available since 2004. We put effort in education, website construction and development of a specific pharmacogenetic request form. Test requests are discussed with the Dept. Pharmacy, and when valid, pharmacogenetic testing is performed under high quality conditions at our department. Test results are discussed with a pharmacist and clinical pharmacologist, and specialized reports are sent out. The turn-around-time at present is 1-2 weeks. To address specific questions regarding genotype and drug dosing, a national task force was installed that systematically reviews the literature, and composes evidence-based dose recommendations. These recommendations are in a national database, which is accessible by all pharmacists in the Netherlands. This ensures widespread and uniform(!) guidelines on how to adjust drug prescriptions based on pharmacogenetic test results.

In our 6 year experience, we noticed some obvious, but also less obvious obstacles for bringing pharmacogenetics to the physician, aiding the individual patient. But one aspect became quite clear: there is a need to obtain additional information in order to optimize drug therapy. It is our challenge to try to find the best way to accomplish this as quickly as possible.

Fitting treatments to patients: implementing personalized healthcare in oncology R&D

Christian Meisel

Roche Pharma Research & Early Development, Penzberg, Germany

It is still clinical reality that patients, who have been diagnosed with the same type of cancer and who receive the same kind of therapy can respond very differently. Whereas some patients experience cure or considerable improvement under therapy, others may benefit only shortly and progress, or do not even respond at all. Although apparently diagnosed with the same disease, patients can today be characterized further by individual traits which are linked to the underlying disease or to individual patient's characteristics.

The goal of personalized healthcare is to identify and define these individual differences in order to be able to optimize therapy, to make healthcare better, safer and more cost-effective. In essence, personalized healthcare (PHC) can be looked at as the attempt to target the right treatment to the right patient in the right manner.

Despite today's molecular insights and technologies to better adjust treatment to the patients, fitting a particular treatment to patients is still an ambitious goal, and discovering and developing novel medicines and diagnostic markers (Biomarkers) is still a very complex and time consuming undertaking.

In this respect Roche is uniquely positioned as a combined pharmaco-diagnostic company to deliver clinically differentiated medicines, and Personalized Healthcare has been adopted to become an integral part of Roche's pharma-diagnostic strategy.

In the presentation, PHC and companion diagnostics examples, with a focus on tumor genetics, from the Roche Oncology pipeline, as well as lessons learnt will be shared.



Invited
Speakers'
Biographies



Magnus Ingelman-Sundberg

*Karolinska Institutet,
Stockholm, Sweden*

Magnus Ingelman-Sundberg serves as Chairman of the Recruitment committee at Karolinska Institutet and is a member of *The Nobel Assembly* at Karolinska Institutet. He is Chairman of the Editorial Board of *Pharmacogenetics and Genomics* and Chairman of the Microsomes and Drug Oxidation organisation. He has 350 original papers, 17 500 citations and an h-factor of 73. Ingelman-Sundbergs group identified the first example of stable gene duplication and gene amplification in the human genome in 1993 and identified the ultrarapid metaboliser (UM) phenotype for CYP2D6 and CYP2C19. The current research is centered about genetic predictors for drug response and adverse drug reactions.



Alex Sparreboom

*St. Jude Children's Research Hospital,
Memphis, USA*

Dr. Sparreboom is an Associate Member in the Department of Pharmaceutical Sciences at St. Jude Children's Research Hospital. He received his BSc, MSc, and PhD in Pharmacy from Utrecht University, and he was previously affiliated with the Daniel den Hoed Cancer Center at Erasmus University Medical Center and with the National Cancer Institute, NIH. Dr. Sparreboom has published over 250 peer-reviewed articles and book chapters, and he is presently Senior Editor of *Clinical Cancer Research* and Associate Editor of *Clinical Pharmacology & Therapeutics*. His research interests focus on the role of solute carriers in anticancer drug disposition and toxicity.



Julian Downward

*Cancer Research UK London Research Institute,
London, UK*

Julian Downward obtained his bachelor's degree from Cambridge University and then his Ph.D. in the laboratory of Michael Waterfield at the Imperial Cancer Research Fund in London, where he established in 1984 the link between a retroviral oncogene (v-erbB) and a cellular growth regulatory protein, the EGF receptor. In 1986, he moved to Robert Weinberg's laboratory at the Whitehead Institute at the Massachusetts Institute of Technology in Cambridge, MA, where he began work on the role of RAS proteins in human cancer. In 1989 he started his own lab at the Cancer Research UK London Research Institute, where he has provided insights into the molecular mechanisms of function and regulation of oncogenic proteins of the RAS family and the importance of their mutational activation in human tumours.

Recently he has focused on oncogene addiction and drug resistance in cancer, developing and applying high throughput functional genomic technologies. He is a Fellow of the Royal Society, the UK's national academy of sciences, and Associate Director of the Cancer Research UK London Research Institute.



Anna Di Rienzo

*The University of Chicago,
Chicago, USA*

Anna Di Rienzo is a Professor of Human Genetics at the University of Chicago and a member of the Committees on Genetics, Genomics and Systems Biology, on Clinical Pharmacology and Pharmacogenomics, and on Molecular Metabolism and Nutrition. She received her BS in Biological Sciences and her PhD in Medical Genetics from the University of Rome "La Sapienza". Her group studies patterns of variation at the DNA sequence and the gene expression levels across human populations with particular interest in genes coding for drug metabolizing enzymes and other genes playing a role in common clinical and non-clinical phenotypes.



Peter Campbell

*Wellcome Trust Sanger Institute,
Cambridge, UK*

Dr Peter Campbell is a Group Leader at the Wellcome Trust Sanger Institute, having started a Wellcome Trust Senior Clinical Fellowship in 2010. He completed specialist training in Haematology in 2002, and has been admitted as a Fellow of the Royal Australasian College of Physicians and the Royal College of Pathologists of Australasia. Following this, he completed a PhD at the University of Cambridge in the molecular pathogenesis and clinical management of myeloproliferative disorders under the supervision of Prof Tony Green. Since 2007, Dr Campbell has been employed at the Cancer Genome Project, Wellcome Trust Sanger Institute, initially on a KKLf Intermediate Clinical Fellowship.

His major interest is cancer genomics, and his recent research has been concentrated on the implementation of next generation sequencing technologies for the detection of somatically acquired genetic variants in tumour samples. One major aspiration is to develop translational applications of high-throughput genomic screening for the care of patients with cancer.



Rafael Rosell

*Catalan Institute of Oncology,
Barcelona, Spain*

Dr Rosell has authored over 500 articles in peer-reviewed journals, some of which are listed below, and given more than 600 presentations at conferences around the world. Since the early nineties, he has been involved in the application of genetic research to the clinical setting. His study examining the role of K-ras mutations was published in *Oncogene* in 1993, and in 1994, his seminal study on neoadjuvant chemotherapy versus surgery alone in locally advanced non-small-cell lung cancer in *The New England Journal of Medicine*. From 1996 to 1998, Dr Rosell's team published papers on microsatellite instability in lung and colon cancer and on H-ras minisatellite alleles in non-Hodgkin's lymphoma. More recent translational research by Dr Rosell's group has focused on chemosensitivity in relation to polymorphisms of DNA repair genes, methylation patterns in circulating DNA, gene expression profiles in RNA, and molecular classification of cancers. Dr Rosell's group has also been involved in research on EGFR mutations and has implemented large-scale screening for EGFR mutations in newly-diagnosed lung cancer patients in Spain in order to select patients for treatment with EGFR inhibitors instead of chemotherapy as first-line treatment.



Manel Esteller

*Bellvitge Biomedical Research Institute (IDIBELL),
Barcelona, Spain*

Manel Esteller (Sant Boi de Llobregat, Barcelona, Catalonia, Spain, 1968) graduated in Medicine with Honours from the Universidad de Barcelona in 1992, where he also obtained his Ph.D. degree specialising in molecular genetics of endometrial carcinoma, in 1996. He was an Invited Researcher at the School of Biological and Medical Sciences at the University of St. Andrews, (Scotland, UK) during which time his research interests focused on the molecular genetics of inherited breast cancer.

From 1997 to 2001, Esteller was a Postdoctoral Fellow and a Research Associate at the Johns Hopkins University and School of Medicine, (Baltimore, USA) where he studied DNA methylation and human cancer. His work was decisive in establishing promoter hypermethylation of tumour suppressor genes as a common hallmark of all human tumours. From October 2001 to September 2008 Manel Esteller was the Leader of the CNIO Cancer Epigenetics Laboratory, where his principal area of research were the alterations in DNA methylation, histone modifications and chromatin in human cancer. Since October 2008, Dr Esteller is the Director of the Cancer Epigenetics and Biology Program of the Bellvitge Institute for Biomedical Research (IDIBELL) in Barcelona and leader of the Cancer Epigenetics Group. His current research is devoted to the establishment of the epigenome maps of normal and transformed cells, the study of the interactions between epigenetic modifications and non-coding RNAs, and the development of new epigenetic drugs for cancer therapy.

Author of more than two hundred-twenty original peer-reviewed manuscripts in biomedical sciences, he is also a Member of numerous international scientific societies, Editorial Boards and reviewer for many journals and funding agencies. Dr Esteller is also Associate Editor for Cancer Research, The Lancet Oncology and Carcinogenesis, Editor-in-Chief of Epigenetics and Advisor of the Human Epigenome Project, Associate Member of the Epigenome Network of Excellence and President of the Epigenetics Society. His numerous awards include: Best Young Cancer Researcher Award bestowed by the European School of Medical Oncology (1999), First Prize in Basic Research at the Johns Hopkins University and Medical Institution (1999), Best Young Investigator Award from the European Association for Cancer Research (2000), Young Investigator Award from the American Association for Cancer Research-AFLAC (2001), Carcinogenesis Award (2005), Beckman-Coulter Award (2006), Francisco Cobos Biomedical Research Award (2006), Fondazione Piemontese per la Ricerca sul Cancro (FPRC) Award (2006), Swiss Bridge Award (2006). National Research Award in Oncology "Maria Julia Castillo" (2007), "Dr Josep Trueta" Award by the Academy of Medical

Sciences of Catalonia (2007), Innovation Award from the Commonwealth of Massachusetts (2007), Human Frontier Science Program Award (2007) "Dr. Jacint Vilardell" Foundation Award (2008), DEbiopharm-EPFL Award (2009), Dr. Josef Steiner Cancer Research Award (2009), Lilly Foundation Preclinical Biomedical Research Award (2009), Fundación Esteve Award (2009), Fundación AECC Award for Children Cancer Research (2009), Carmen y Severo Ochoa Foundation, Molecular Biology Research Award (2009), Caja Rural Granada Foundation Health Science Award (2010) and World Health Summit and Pfizer Award for Innovation in Biomedical Research (2010).

Dr Manel Esteller is the Director of the Cancer Epigenetics and Biology Program of the Bellvitge Institute for Biomedical Research (IDIBELL), Leader of the Cancer Epigenetics Group, Professor of Genetics in the School of Medicine of the University of Barcelona, and an ICREA Research Professor.



William E. Evans

*St. Jude Children's Research Hospital,
Memphis, USA*

Dr. William E. Evans is Director and Chief Executive Officer of St. Jude Children's Research Hospital (SJCRH), and holds the St. Jude Professorship and Endowed chair at the University of Tennessee Colleges of Medicine and Pharmacy.

For the past 30 years, his research at St. Jude has focused on the pharmacogenomics of anticancer agents in children, for which he has received three consecutive NIH MERIT Awards from the National Cancer Institute (1987-2015). The major disease focus of his pharmacogenomics research is acute lymphoblastic leukemia in children.

He is a member of the Board of Trustees of Rhodes College, the Board of Directors of Methodist Le Bonheur Healthcare, the Board of Directors of the Memphis Chamber of Commerce, the Board of Memphis Tomorrow and is Chair of the Board of Directors of the Tennessee Technology Development Corporation. He currently serves on several Scientific Advisory Boards including the Board of Scientific Counselors for the US National Cancer Institute of NIH.

Dr. Evans has authored over 300 articles and numerous book chapters, has been the editor of several textbooks and scientific journals. He has received several national awards for his research, including the 2009 Pediatric Cancer Award from the American Society of Clinical Oncology (shared with Mary V. Relling of SJCRH) and the 2009 Team Science Prize from the American Association of Cancer Research (shared with his Hematological Malignancies colleagues at St. Jude). He is recognized by ISI as a "Highly Cited Scientist" in pharmacology, based on citations of his research publications. He was elected to the Institute of Medicine of the National Academy of Sciences in 2002.



Howard McLeod

*University of North Carolina,
Chapel Hill, USA*

Dr Howard McLeod is Fred N. Eshelman Distinguished Professor and Director, UNC Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina, Chapel Hill. Dr McLeod holds appointments in the Schools of Pharmacy and Medicine, the Carolina Center for Genome Sciences, and the Lineberger Cancer Center. Dr McLeod is chair of the NHGRI eMERGE network external scientific panel and is a member of the FDA committee on Clinical Pharmacology. He also directs the Pharmacogenetics for Every Nation Initiative, which aims to help developing countries use genetic information to improve National Drug Formulary decisions. Howard has published over 380 peer reviewed papers on pharmacogenomics, applied therapeutics, or clinical pharmacology and continues to work to integrate genetics principles into clinical practice to advance individualized medicine.



Anna González-Neira

CNIO,
Madrid, Spain

Anna González-Neira studied at the Universidad Complutense de Madrid, where she graduated in Biology in 1995. In 1996 she started working at the Faculty of Medicine in the Universidad de Santiago de Compostela (Spain), from where she obtained her PhD degree in Medicine under the supervision of A. Carracedo (Thesis Prize from Universidad Santiago de Compostela). During this period she was also a Research Fellow at the Office of the Chief Medical Examiner, Department of Forensic Biology, New York University Medical Centre (USA) and at the Institute of Pathology and Molecular Immunology (IPATIMUP), Universidade do Porto, Portugal.

She spent three years as a Postdoctoral Fellow at the Unit of Evolutionary Biology, Universidad Pompeu Fabra in Barcelona under the supervision of J. Bertranpetit. She has also held fellowships at the Wellcome Trust Sanger Institute, Cambridge (UK) in the groups of I. Dunham and P. Deloukas. She later moved to Oxford (UK) to work at the Department of Bioinformatics and Statistical Genetics, Wellcome Centre of Human Genetics, supervised by L. Cardon. She joined the CNIO in September 2004 to head the Genotyping Unit.

Her current research activity focuses on the identification of the genetic variations in humans responsible for differences in cancer susceptibility and response to anticancer drugs.



Cristina Rodríguez-Antona

CNIO,
Madrid, Spain

Cristina Rodríguez-Antona is graduated in Chemistry in the University Complutense de Madrid and has a Diploma in Biological Chemistry from the University of Kent at UK. In 2001 she obtained a European PhD with First Class Honours from the University de Valencia, after short-term stays at the Fraunhofer Institut für Toxikologie und Aerosolforschung (1998, Hannover), CNRS (1999, Montpellier) and Karolinska Institute (2001, Stockholm).

She was awarded a Marie Curie Grant to carry her postdoctoral studies in the laboratory of M. Ingelman-Sundberg at the Institute of Environmental Medicine of the Karolinska Institute, Stockholm, Sweden, where she worked between 2001 and 2005. During this period she mainly focused on cytochrome P450 enzymes and through Pharmacogenetic approaches identified novel clinically relevant genetic variants altering drug response.

In 2005 she was awarded a Ramon y Cajal and a Marie Curie Reintegration Grant and joined the Hereditary Endocrine Cancer Group at CNIO. Over the past few years her research has been focused on the identification of genetic predictors of anti-cancer drug treatment response. To address this challenge she is using classical as well as full genomic approaches together with *in vitro* functional assays. She is currently principal investigator of several pharmacogenomic projects focused on cancer treatment and has established important collaborations with Clinical Oncology Units.



Jörg D. Hoheisel

*German Cancer Research Center (DKFZ),
Heidelberg, Germany*

Jörg D. Hoheisel is Head of the *Division of Functional Genome Analysis* and Chairman of the Scientific Council of the Deutsches Krebsforschungszentrum (DKFZ; German Cancer Research Centre) in Heidelberg, Germany. Prior to joining DKFZ in 1993, he worked for five years in the group of Hans Lehrach at the Imperial Cancer Research Fund in London, UK. Before, he had been trained as a molecular biologist at the University of Constance, Germany, where he also did his diploma graduation and Ph.D. degree with Fritz M. Pohl.



Javier Benítez

*CNIO,
Madrid, Spain*

Javier Benitez, PhD in Human Genetics, Madrid (1982). He spent several years at the Fundación Jimenez Díaz. (Madrid) where he was Head of the Human Genetics Service. He moved to CNIO in 2000 as Director of the Human Genetics Dptm and in 2005 he was appointed as Director of the Human Cancer Genetics Program. He has been President of the Spanish Society of Human Genetics, Professor of Human Genetics at the Fco Victoria University (Madrid) and author of more than 230 international publications. He is also Director of the Spanish National Genotyping Centre at Madrid. His current work is focussed in the study of familial cancer and the development of high throughput technologies.



Arcadi Navarro

*Pompeu Fabra University,
Barcelona, Spain*

Arcadi Navarro was an undergraduate, and later a graduate student, at the Universitat Autònoma de Barcelona, where he started his PhD in Biology in 1993. After quitting the academic world for a few years, he went back to basic research in 1999 as a postdoctoral fellow at the University of Edinburgh. In 2002 he entered the Universitat Pompeu Fabra (UPF) as a researcher within the Ramón y Cajal program and was appointed ICREA Research Professor at the UPF in November 2006. Since 2009 he is also Professor of Genetics in that department. Currently he leads a research group in Evolutionary and Population Genomics within the Department of Experimental and Health Sciences of the UPF. In addition, he is the director of the Population Genomics Node of the Spanish National Institute for Bioinformatics (INB) and the Vice-director of the Institute for Evolutionary Biology (IBE), a recently created joint institute between the UPF and the Consejo Nacional de Investigaciones Científicas (CSIC).



Miguel Martín

*Gregorio Marañón Hospital,
Madrid, Spain*

Dr. Miguel Martín, born in 1954, is currently Head of the Medical Oncology Service at the Hospital General Universitario Gregorio Marañón in Madrid (Spain). He is also a Professor of Medical Oncology at the Complutense University of Madrid and the Chairman of GEICAM (Spanish Group for Breast Cancer Research), a cooperative network involving more than 150 Spanish institutions.

Dr. Martín studied Medicine at the University of Valladolid (Spain) between 1971 and 1977. From 1980 to 1983 he did his Residence in Internal Medicine/Medical Oncology, at the Hospital Universitario San Carlos in Madrid (Spain). In 1984, he got the Spanish Certification on Medical Oncology and in 1985, a Ph.D. degree. In 1989, he obtained the European Certification on Medical Oncology. From 1983 to 1991, Dr. Martín worked as Attending Physician in the Medical Oncology Department, at the University Hospital in Madrid (Spain). From 1991 to 1992, he visited the Fred Hutchinson Cancer Research Center and the University of Washington Medical Center in Seattle (USA). In 1994, he was appointed as Chief of Breast Cancer Section in the Medical Oncology Department, at the University Hospital in Madrid (Spain).

Dr. Martín is a founder member of CIRG (Cancer International Research Group) and serves a member of the Board of Directors of CIRG. He also is member of the Steering Committee of the CIRG Breast Cancer Working Group. Dr. Martín is a former member of the International Affairs Committee of ASCO (American Society of Clinical Oncology).

Dr. Martín is the first author of more than 100 original articles in Spanish and international journals, including New England Journal of Medicine, Journal of the National Cancer Institute, Journal of Clinical Oncology, Lancet Oncology, Annals of Oncology, Breast Cancer Research and Treatment, Seminars in Oncology, and others, mainly devoted to breast cancer, new drugs and supportive care. He has also co-authored other 120 articles in Spanish and international journals. He is an active member of the following Societies: ASCO, ESMO, American Society of Breast Diseases, EBMT, ABMT and MASCC.



Ron Van Schaik

*Erasmus University Medical Center,
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Ron van Schaik (PhD, Clinical Chemist and Associate Professor Pharmacogenetics) works at the Dept. Clinical Chemistry at the Erasmus University Medical Center Rotterdam as Coordinator Specialized Research & Development and Director of the Pharmacogenetics Core Laboratory. Dr. van Schaik leads a research group on pharmacogenetics, focussing on translation and implementation into patients diagnostics. Research concentrates on transplantation/immunosuppression, oncology, pain and HIV drugs. He published over 95 articles on pharmacogenetics, and participates in international advisory committees (a.o. IFCC Task Force Pharmacogenetics (Chair), IATDMCT Pharmacogenetics Committee (chair), Dutch Task Force Pharmacogenetics (chair), European Pharmacogenetics Research Network (Steering Committee)). In 2001, he received the Ortho Clinical Diagnostics Award for outstanding research. In 2008, his laboratory was internationally recognized as IFCC Reference Laboratory for Pharmacogenetics.



Christian Meisel

*Roche Pharma Research & Early Development,
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Christian is a physician scientist, board-certified both in internal medicine and clinical Pharmacology. After his academic career, where Christian served as Associate Professor, and Head of the drug therapy information and consultation service of Charité University in Berlin, as well as the Scientific Managing Director and Head of the information center of the Research Alliance "Pharmacogenetics and pharmacogenetic diagnostics: improving therapy and drug development", he joined Roche Headquarters in Basel where he was initially responsible for the medical genetics strategies of the Roche development compounds. In 2006, Christian moved to Roche Penzberg, where he took over global responsibility for Oncology Biomarkers. Since 2010, Christian is the Oncology Site Leader, as well as the Site Head Translational Medicine in Penzberg.



Poster
Session

DNA repair pathway related polymorphisms in the onset of oxaliplatin neuropathy after adjuvant FOLFOX

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Oxaliplatin is used in the adjuvant treatment of colorectal cancer (CRC). Its dose limiting side effect is a cumulative peripheral neuropathy usually observed after 4 to 6 months of treatment. DNA repair pathway efficiency could be associated to the molecular mechanism of oxaliplatin-related neurotoxicity. Aim of this prospective study is to define the role in the neurotoxicity development of a panel of genetic polymorphisms in the DNA repair pathway. We analyzed 23 polymorphisms in 15 genes (XPD, XRCC1, XRCC3, APE1, hOGG1, hMSH6, hMSH2, hMLH1, PARP1, MGMT, hEOX1, ERCC1, RAD51, XPG, ATM), in 154 CRC patients homogeneously treated with the adjuvant FOLFOX regimen (oxaliplatin, 100 mg/m² every 2 wks+ 5-fluorouracil/leucovorin). Neuropathy was graded according to the oxaliplatin-specific scale (by Caussanel). Fisher's Exact test and stepwise logistic regression analysis were employed for the calculation of neurotoxicity relationships to the polymorphisms. The molecular analyses, based on Pyrosequencing[®] and allelic discrimination by TaqMan technologies, were carried out on genomic DNA extracted from peripheral blood lymphocytes. Univariate analysis highlighted a significant association between severe grade 3 neuropathy and two polymorphisms: XRCC3-rs1799796 and APE1-rs1130409 that seem affect the DNA repair capacity in response to oxaliplatin induced damage. Both of them represent risk factors for neurotoxicity development after oxaliplatin administration. Patients bearing at least one variant XRCC3- rs1799796 allele had a higher chance to get grade 3 neurotoxicity (OR=5.00 95%CI 1.37- 18.29; P=0.021). Patients with homozygous APE1-rs1130409 variant genotype had a higher chance to get grade 3 neurotoxicity (OR=3.54 95%CI 1.00- 12.64; P=0.048). These results are intended to be replicated in an independent validation set of patients. In conclusion, two polymorphisms in the DNA repair pathways (XRCC3-rs1799796 and APE1-rs1130409) could be useful in selecting patients at risk for G3 neuropathy development from the FOLFOX regimen used as adjuvant chemotherapy in CRC.

Genetic variation in SLC19A1 gene influences methotrexate toxicity in rheumatoid arthritis patients

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Methotrexate (MTX) is a folate antagonist widely used for cancer and rheumatoid arthritis (RA) treatment. MTX enters the cells through the reduced folate carrier SLC19A1. Several studies have investigated the association of SLC19A1 SNPs, mainly rs1051266 (80G>A; R27H) and rs1131596 (-43T>C), with MTX treatment outcome, however, conflicting results have been reported.

Our aim was to perform a comprehensive study, using both individual SNPs and haplotypes of the SLC19A1 gene, to elucidate the effect of SLC19A1 genetic variation on the occurrence of MTX adverse events and MTX-induced treatment discontinuation. Methods: Two putatively functional SNPs in high linkage disequilibrium rs1051266 and rs1131596, and five SLC19A1 tagging SNPs (rs2838951, rs2838956, rs2838958, rs2838451 and rs17004785) were genotyped in 212 unrelated RA patients treated with MTX and the genotypes compared with clinical outcome. SLC19A1 mRNA levels were quantified by qRT-PCR in lymphoblastoid cell lines from 89 HapMap individuals.

Multivariate analysis of the discontinuation of MTX treatment due to toxicity showed that rs1051266 and rs1131596 were associated with protection (HR per allele =0.46, 95%CI=0.30-0.83, P=0.010 and HR=0.47, 95%CI 0.26-0.84, P=0.011, respectively) and rs2838956 with a protection trend (P=0.077). In addition, significant associations were also found for these SNPs with skin adverse effects and infections. From the two haplotypes carrying rs1051266 and rs1131596 minor alleles, the less common one was significantly associated with protection towards MTX discontinuation due to toxicity (P=0.025), while the most common one showed a trend towards protection (P=0.080). Quantification of SLC19A1 mRNA levels in lymphoblastoid cell lines suggested that rs1131596 was not a major causal variant underlying the observed associations.

Individual SNP and haplotype analyses suggest that rs1051266 is a functional variant associated with MTX toxicity. Prospective studies with large sample sizes are needed to further support these results.

DNA repair functionality and response to trabectedin in breast cancer

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Trabectedin (Yondelis®, ET-743) is a marine-derived compound discovered in the tunicate *Ecteinascidia turbinata* that present antitumor activity and is approved in Europe and other countries for use in patients with advanced previously treated sarcomas and in ovarian cancer. A composite signature including low *BRCA1* and either high *ERCC1* and/or *ERCC5* identifies a highly sensitive population of sarcomas with significantly improved treatment outcome. *BRCA1* hereditary breast tumours present *BRCA1* loss of function and in about 15% of familial *BRCAX* and sporadic breast tumours would present somatic inactivation of *BRCA1* (*BRCA1*-like). A subset of these tumours that in addition present high levels of expression of *ERCC5/ERCC1* might be particularly sensitive to Trabectedin and this could have potential impact on strategies to individualize treatment of breast cancer.

We have characterized levels of expression of DNA-repair genes belonging to the HR (*BRCA1, BRCA2* and *XRCC3*) and NER (*ERCC1, ERCC5, CUL4A*) pathways in 50 sporadic, 67 familial breast tumours and 11 breast cancer cell lines by qRT-PCR. We have estimated the frequency in which the “target” signature (impaired HR/Efficient NER) is found in the primary breast tumours and characterized Trabectedin response in the panel of cell lines.

All breast cancer cell lines analyzed were highly sensitive to Trabectedin treatment. Main effect after Trabectedin exposure was cell growth inhibition followed by apoptosis. Positive correlation between IC50 values and *BRCA1/ERCC5* expression ratio suggests that predictive signature of Trabectedin sensitivity might be valid not only in sarcomas but also in breast cancer. If so, according to our gene expression screening, up to 25% of sporadic and familial breast tumours would exhibit the low *BRCA1/high ERCC5* signature for Trabectedin sensitivity. However, we have found that high *CUL4A* expression may be a better predictive indicator of sensitivity to Trabectedin, either alone or in combination in low *BRCA1* expression.

Immunohistochemical characterization of sporadic ovarian serous carcinomas vs non-serous carcinomas. Clinical implications

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Introduction: Ovarian cancer (OC) is the leading cause of death from gynecological malignancy and is the fourth cancer-related cause of death among women. Due to absence of early symptoms and to the inadequacy of available screening methods, OC are often diagnosed at an advanced stage resulting in a low survival rate. The standard treatment of both early and advanced epithelial ovarian cancer is primary debulking surgery followed by combination chemotherapy with addition of a taxane to a platinum-based regimen. This treatment improves survival in patients with epithelial ovarian cancer and the initial response rates are as high as 70% to 85%. However, an unfortunate reality is that despite this initial high response rate, most of these patients will experience recurrence of the cancer, with small chance of cure. Therefore, the development of predictive markers that will guide treatment decisions and novel effective targeted therapies are urgently required in this disease. Aims: 1) To perform an immunohistochemical analysis (IHQ) of sporadic epithelial ovarian tumours to establish a molecular classification; 2) To find predictive markers of treatment resistance/respond; 3) To identify candidate markers of targeted therapies. Material/methods: We have compiled 84 sporadic ovarian carcinomas (40 serous and 44 nonserous carcinomas) from Hospital Virgen del Rocío (Sevilla). Two Tissue Microarrays have been built to carry out an IHQ analysis of 27 antibodies (cell cycle, apoptosis, proliferation, hormonal, adhesion molecules, DNA repair and targeted therapies markers) and histological study. To obtain clinicopathological correlations we have also elaborated a clinical form with data about outcome and treatment of patients. Results: Analysis of clinical features reveals that serous carcinomas (SC) have a higher recurrence rate, volume of residual disease after surgery, and reduced progression free interval and overall survival than non-serous carcinomas (NSC) (P=0.002; P=0.005; P=0.009; P=0.033, respectively). From histopathological point of view, SC are neoplasms that are diagnosed at more advanced stage, bilateral and have a higher grade than NSC (P=0.031; P=0.003; P=0.002, respectively). Regarding to IHQ comparative analysis most relevant results are a higher expression of estrogen receptor (ER), ERCC1, XPF, Ki-67, P53 and Cyclin E in SC than NSC group (P<0.001; P=0.017; P=0.035; P=0.007, P=0.01 and P=0.005, respectively). In addition, SC tend to have a higher expression of progesterone receptor (PR) than NSC (P=0.09). By contrast, there is a higher proportion of NSC that have nuclear expression of beta catenin and overexpress beta tubulin III (P<0.006 and P=0.045). Conclusions: 1) From clinicopathological point of view, SC are more aggressive neoplasms than NSC; 2) Due to the higher expression of ERCC1 in SC, they are more platinum resistant than NSC; 3) Higher expression of ER and trend of overexpression of PR in SC suggest that hormonal therapy could be more useful in serous group than non-serous; 4) NSC have a greater proportion of beta catenin mutation and they are more taxane resistant (overexpression of beta tubulin III) than serous group.

A Let-7 microRNA complementary site polymorphism in the KRAS 3'-UTR region as a genetic regulator in advanced colorectal cancer

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KRAS status has been identified as a strong predictor of resistance to anti-EGFR therapies, however not all wild type patients respond. The microRNAs, highly conserved small non-coding RNAs, regulate gene expression at the posttranscriptional level through binding to the 3'-untranslated region (UTR) of the target mRNAs. Let-7 family of microRNAs regulates KRAS expression and has been associated with colorectal cancer outcome. The fact that the functional KRAS-LCS6 variant affects the KRAS expression led us to hypothesize a possible association between the KRAS-LCS6 polymorphism and the response to anti-EGFR treatments.

In this study were included 91 patients with KRAS wild type metastatic colorectal adenocarcinoma treated with anti-EGFR antibodies in monotherapy or in combination with chemotherapy. We genotyped all patient samples for the KRAS Applications LCS6 polymorphism using the 48.48 dynamic array chips on the BioMark™ system (Fluidigm)

Seventy seven patients presented LCS6 T/T genotype (85%) while 14 were T/G and G/G (15%). Two patients (2%) had CR, 20 had PR (22%), 36 had SD (40%) and 26 progressed (29%). LCS6 G-allele showed statistically significant association with non-response; 31% of patients with T/T genotype presented CP or PR vs no patients with T/G or G/G genotypes ($p=0.031$). Multivariate analysis confirmed that KRAS-LCS6 polymorphism and skin toxicity were independently related with response ($p<0.05$).

Presence of the LCS6 G-allele can predict clinical response to anti-EGFR treatment in patients with KRAS wild type metastatic colorectal adenocarcinoma.

A genome-wide study to identify genetic variants associated with cisplatin-induced hearing loss

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Osteosarcoma is the most frequent malignant bone tumor in children and adolescents. Standard treatment for osteosarcoma includes neoadjuvant therapy with a combination of cisplatin, adriamycin and methotrexate before surgical resection of the primary tumour, followed by postoperative chemotherapy. However, a significant group of patients relapse after treatment or develop severe adverse events. One of the main adverse events in the treatment of osteosarcoma patients is ototoxicity, which is related to cisplatin treatment that leads to hearing loss and has a profound impact especially in children. In order to identify genetic variants associated with cisplatin-induced ototoxicity, we performed a genome-wide association study (GWAS) in 48 cisplatin-treated osteosarcoma patients. To this purpose, we genotyped approximately 660000 SNPs using the Illumina Human 660-Quad chip using DNA from peripheral blood samples. Hearing loss was evaluated by audiometric tests at the otorhinolaryngology consultation after treatment with cisplatin. We studied the association of the genotypes with ototoxicity by logistic regression analysis. Data are currently being analyzed in order to identify new genetic variants capable of predicting ototoxicity. A replication cohort is being collected in order to validate the most significant results. The identification of new genetic markers that contribute to cisplatin ototoxicity could improve the treatment for osteosarcoma patients, helping in the design of individualized therapy.

Pharmacogenetics of childhood acute lymphoblastic leukemia

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Acute lymphoblastic leukaemia (ALL) is the most common malignancy in childhood, accounting for 30% of all cancers in children. Treatment outcome has steadily improved due to the optimal use of antileukemic agents developed from the 1950s to the 1980s, and the stringent application of prognostic factors for risk-directed therapy in clinical trials. Despite such advances, however, almost 20% of the children either relapse or do not respond to treatment. This seems to be related to parameters such as the presence of genetic polymorphisms that influence treatment efficacy and toxicity. We analyzed the association of polymorphisms in MTHFR, DHFR, RFC1, TS and CCND1 genes on therapy-related toxicity and survival in 133 ALL children treated with methotrexate in the consolidation period of their treatment. The MTHFR alleles associated with a reduced enzymatic activity were found to be highly associated with nephrotoxicity ($p < 0.05$). The group of patients carrying MTHFR alleles associated with a normal enzymatic activity were treated with higher doses of methotrexate (5 vs 3 g/m²) and a clear improvement in the disease-free survival was observed (44,8 months vs 36,0 months; $p < 0.05$). Our results support the idea that the MTHFR genotype may help in individualising methotrexate dosing in childhood ALL therapy.

Selected genetic variants on interferon stimulated genes are related to treatment outcome of patients with chronic hepatitis C

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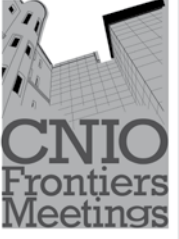
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Pegylated-interferon-alpha (PEG-IFN) plus ribavirin is the most effective therapy for individuals chronically infected by hepatitis C virus (HCV). While patients with virus genotypes (VG) 2 and 3 show the highest rate of sustained virological response (SVR), the infection with VG 1 has only a successful response in a 40% of patients. PEG-IFN treatment triggers the antiviral immune response by its binding to the interferon-alpha receptor which mediates the expression of the interferon stimulated genes (ISGs) through the JAK-STAT pathway. The aim of the present work was to elucidate whether the genetic variants in proteins implicated in this pathway may contribute to the antiviral immune response outcome. 283 patients with chronic hepatitis C (CHC), 226 VG 1 and 57 VG not 1, treated with PEG-IFN plus ribavirin were included after gave their written consent. Response to treatment was defined as sustained virological response (SVR) or no response (NR) following current practice guidelines. A total of 69 polymorphisms of 30 genes implicated in the IFN signaling (IFNAR1-2, JAK1, TYK2, STAT1-2, OAS1-3, OASL and RNASEL among others) were genotyped using the Illumina GoldenGate Genotyping Assay. Statistic association with SVR was determined by logistic regression adjusted by VG. Those SNPs significantly associated with SVR ($p < 0.05$) were further investigated after being adjusted by other covariates such as age, sex and basal levels of ALT, AST, GGT and pre-treatment viral load (SPSS 15.0). Finally, haplotype association of these polymorphisms with SVR was also assessed (SNPstats, ICO). A total of 13 polymorphisms in IFNAR1 (rs2834202), STAT1 (rs2030171), OAS1 (rs2057778, rs7135577, and rs1051042), OAS2 (rs1293764), OAS3 (rs10735079), OASL (rs12819210), RNASEL (rs12135247 and rs1048260), IFIT1 (rs304478 and rs303217) and ICAM (rs7257871) genes showed differences in the rate of SVR after adjusting by GV ($p < 0.05$). When other covariates were taken into account by the logistic regression analysis, VG, age and basal GGT were significantly associated to the SVR ($p < 0.05$), as well as the SNPs rs12819210, rs1051042 and rs304478 ($p < 0.05$). Noteworthy, OAS1 haplotype analysis [rs2057778 (A-C), rs1051042 (C-G) and rs7135577 (A-G)] showed a lower SVR associated to CGA haplotype compared to the most common haplotype (ACG) in our patient population ($p < 0.05$). Our data shows a significant association of some ISGs SNPs with the achievement of the sustained virological response to current CHC antiviral treatment. Higher sample size could be helpful to better precise these results.



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28029 Madrid, Spain

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Coordination and edition: Mercedes Moro and Carolina G. Sabaté, CNIO

Direction of art and production: Versus Gráficas S.A.

Photographic archive: CNIO

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