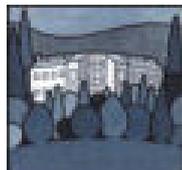




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## **III CONVEGNO MONOTEMATICO SIF**



**FARMACOGENOMICA E CANCRO:  
DAL LABORATORIO ALLA CLINICA**

**8 OTTOBRE 2011**

**GRADO (GO)  
GRAND HOTEL ASTORIA**



**SOCIETÀ ITALIANA FARMACOLOGIA**

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## **BENVENUTI a Grado!**

*La ricerca farmacologica svolge un ruolo determinante per contribuire alla salute e al benessere dell'umanità. La scoperta di nuovi farmaci e lo studio dei meccanismi con cui interagiscono con i complessi sistemi fisiologici e patologici dell'organismo hanno permesso e continuano a promettere di affrontare la terapia con mezzi sempre più appropriati. L'evoluzione della farmacologia, di concerto con le continue scoperte della biologia dell'uomo, ha portato allo sviluppo della farmacogenetica, una nuova branca della ricerca farmacologica che affronta le diversità biologiche, che si traducono in variazioni fisiologiche e patologiche, per trovare la risposta all'uso più razionale dei farmaci. E' questo un grande sforzo cui si stanno dedicando molti giovani ricercatori sia nel campo delle ricerche di base sia in quello delle applicazioni cliniche. Questa ricerca è di fondamentale importanza per permettere ai farmaci già in uso di trovare una utilizzazione funzionale più adeguata. Per quel che riguarda i farmaci "nuovi", potranno essere disegnati su bersagli più specifici e selettivi, con le evidenti ricadute di un sicuro miglioramento della loro efficacia e riduzione degli effetti collaterali, e con una netta riduzione della spesa farmaceutica. Oggi, a Grado, discutiamo del ruolo della farmacogenetica nella terapia dei tumori, certamente una delle patologie più difficili che la terapia farmacologica si trovi ad affrontare. Lo facciamo dando voce ai giovani ricercatori che investono il proprio sapere e dedicano la loro passione nello studio degli aspetti cinetici e dinamici dei farmaci antitumorali. Ringrazio coloro che hanno accettato di venire a condividere le proprie esperienze in questa difficile opera, augurando a tutti un soggiorno a Grado sereno e proficuo.*

Gianni Sava





## **RINGRAZIAMENTI**

Il III Convegno monotematico SIF “FARMACOGENOMICA E CANCRO: DAL LABORATORIO ALLA CLINICA” è stato realizzato con il contributo di:



**Dipartimento di Scienze della Vita, Università degli Studi di Trieste**



## PROGRAMMA

- 08:30 – 9:00: Apertura del meeting e saluti delle Autorità
- Sessione 1: Progettazione e sviluppo preclinico**
- Moderatore: Armando Genazzani (Università del Piemonte Orientale)
- 9:00 – 9:30 **“Farmacogenomica nello sviluppo di nuovi farmaci antitumorali: single target vs multi-target”**  
Silvana Canevari, Istituto Nazionale dei Tumori, Milano.
- 9:40 – 10:40 Comunicazioni libere:
- 9:40 – 10:00 Eva Dreussi  
*“microRNA and pharmacogenetics: focus on neoadjuvant treatment for rectal cancer”*
- 10:00 – 10:20 Paola Poma  
*“Restoration of Raf-1 Kinase Inhibitor Protein levels as a possible therapeutic approach in the hepatocellular carcinoma”*
- 10:20 – 10:40: Sara De Iudicibus  
*“Genetic predictors of glucocorticoid response in paediatric patients with inflammatory bowel diseases”*
- 10:40 – 11:20 Coffee Break
- 11:20 – 12:00 Comunicazioni libere:
- 11:20 – 11:40: Valentina Boscaro  
*“Knock in of cancer mutations in human cells predicts pharmacological response to targeted therapies”*
- 11:40 – 12:00 Gabriele Stocco  
*“Genome-wide identification of determinants of leukaemia cell sensitivity to mercaptopurine”*
- 12:00 – 13:00 Pranzo
- Moderatore: Giuseppe Toffoli (CRO Aviano)
- 13:00 – 13:50 Keynote lecture: **“Pharmacogenomics and the Pediatric Cancer Genome Project”**  
William Evans, St. Jude Children’s Research Hospital, Memphis, USA
- 13: 50 – 14:30 Discussione dei poster a cura di Mary Relling (St. Jude Children’s Research Hospital, Memphis, USA)
- 14:30 – 14:50 Coffee Break

**Sessione 2: Farmacogenomica nei Trial Clinici**

Moderatore: Enrico Mini (Università di Firenze)

14:50 – 15:20 **“Targeted therapy, proof of principle e ruolo dei marcatori”**

Federica Di Nicolantonio (Università di Torino)

15:20 – 16:40 Comunicazioni libere:

15:20 – 15:40 Gloria Ravegnini

*“Association between genotypes and response to the treatment in a subset of previously untreated chronic myeloid leukemia patients”*

15:40 – 16:00

Raffaella Franca

*“Childhood acute lymphoblastic leukaemia (ALL): role of polymorphisms in the GST- $\mu$ , GST- $\theta$  and ABCC1 genes on relapse”*

16:00 – 16:20

Paola Biason

*“Pharmacogenetics in osteosarcoma: the role of nucleotide excision repair gene variants in survival after neoadjuvant chemotherapy”*

16:20 – 16:40

Eva Cuzzoni

*“Role of GST-M deletion in azathioprine metabolism in pediatric patients with IBD”*

16:40 – 17:00

Chiusura del Convegno

# ***Abstracts***

## **Pharmacogenetics in osteosarcoma: the role of nucleotide excision repair gene variants in survival after neoadjuvant chemotherapy**

Paola Biason<sup>1,2</sup>, Chiara Zanusso<sup>1</sup>, Silvia Boffo<sup>1</sup>, Elena De Mattia<sup>1</sup>, Erika Cecchin<sup>1</sup>, Claudia Maria Hattinger<sup>3</sup>, Massimo Serra<sup>3</sup> and Giuseppe Toffoli<sup>1</sup>.

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Osteosarcoma, despite being the eighth most common cancer of childhood, is classified as a "rare disease" representing 2.4% of all malignancies in pediatric patients and approximately 20% of all bone cancers. Among biological markers, previous studies have identified various factors which appeared to be associated with poor prognosis for patients with osteosarcoma (i.e. overexpression of MDR1/P-glycoprotein, P53 gene alteration and translocation). However, amelioration of the prognosis of osteosarcoma should involve molecular approaches to offer patients additional, possibly tailored, therapies.

The nucleotide excision repair (NER) pathway genes code for proteins removing a wide range of DNA lesions including cross-links. A genetic deficiency in DNA repair capacity of NER pathway genes of the xeroderma pigmentosum (XP) and excision repair cross-complementation (ERCC) families could be implicated in the tumorigenesis and response to DNA damaging therapies, such as cisplatin-based neoadjuvant therapy.

In this study, we were able to collect a series of 130 osteosarcoma patients, with primary high-grade osteosarcomas located at the extremities, without metastasis at diagnosis, and all treated with neoadjuvant chemotherapy based on the administration of cisplatin in association with doxorubicin, high-dose methotrexate and ifosfamide. We aim to investigate the role of a common polymorphisms in the NER pathway genes (XP and ERCC) in the tumorigenesis of osteosarcoma and in the response to DNA damaging therapies (in terms of event free survival, EFS): XPD (rs13181 - 35931A>C and rs1799793 - 23591G>A), XPG (rs17655 - 3507G>C), and ERCC1 (rs3212986 - 8092C>A and rs11615 - 19007C>T). Association with event free survival (EFS) were analysed using Kaplan-Meier plots and log-rank test.

A positive association was observed between both XPD SNPs and an increased EFS (HR= 0.34, 95% CI 0.12-0.98 and HR= 0.19, 95% CI 0.05-0.77, respectively). Moreover, the effect appears to be even stronger after combining these two polymorphisms, as none of the seven homozygous variant patients for both SNPs relapsed compared to 24 (58.5%) patients without any variant allele after 60 months of follow up. However, considering that these two XPD SNPs are not in linkage disequilibrium, the multivariate analysis including both SNPs is suggestive of XPD rs1799793 being the main SNP driving these associations. We had also performed a case-control study for relative risk to develop osteosarcoma. Patients carrying at least one variant allele of XPD rs1799793 had a reduced risk of developing osteosarcoma compared to wild type patients (OR=0.55, 95% CI 0.36-0.84).

The identification of genetic markers, which have prognostic value or are predictive of neoadjuvant chemotherapy response, would represent an important tool to reach informed decisions on how to select the subgroup of osteosarcoma patients who are likely to benefit from a more specific, tailored treatment. Based on the results of our study, we propose that XPD rs1799793 is a germline variant to be subsequently tested prospectively in patients with high-grade osteosarcoma.



## From bench to bedside: a genotype-guided phase I study of FOLFIRI and bevacizumab in advanced colorectal cancer patients

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A. Buonadonna<sup>1</sup>, G.M. Miolo<sup>1</sup>, A.M. Colussi<sup>1</sup>, E. Turchet<sup>1</sup>, P. Biason<sup>1</sup>, C. Zanusso<sup>1</sup>,  
E. Mazzega<sup>1</sup>, P. Giusti<sup>2</sup>, G. Toffoli<sup>1</sup>

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Irinotecan (CPT-11) plus 5-fluorouracil (5-FU) and leucovorin (LV) became the standard first-line chemotherapy for colorectal cancer in the U.S. and Europe in 2000. One of the regimens for CPT-11 plus infusional 5-FU/LV therapy is the FOLFIRI regimen. FOLFIRI, is an internationally accepted standard chemotherapy for metastatic colorectal cancer.

Irinotecan is a semisynthetic derivative of the natural alkaloid camptothecin that acts as a prodrug generating *in vivo* the active metabolite, SN-38, through the carboxylesterase activity. After formation, SN-38 can be inactivated by conjugation with glucuronic acid forming SN-38 glucuronide (SN-38G) through an enzymatic reaction UGT1A1 mediated.

Recently FDA approved bevacizumab in combination with fluoropyrimidine-based chemotherapy for first-line treatment of patients with metastatic carcinoma of the colon or rectum. The addition of bevacizumab as an intravenous infusion in combination with irinotecan, 5-fluorouracil and leucovorin (FOLFIRI) has been found to increase the response rates, extend median overall survival and prolong the duration of response by comparison with FOLFIRI alone. The addition of bevacizumab in the FOLFIRI regimen is effective and generally well tolerated however its use is associated with some toxicities.

Inter-patient variability still remains the major concern in response and toxicity of irinotecan treatment. Recent molecular profiling technologies, including genomic/genetic testing, have allowed the development of “personalized medicine”. Irinotecan is one of the models for personalized therapy based on pharmacogenetics. Several clinical studies revealed significant associations between UGT1A1\*28 and irinotecan toxicity. In particular, UGT1A1\*28 (alias TA in/del) polymorphism, characterized by an extra TA repeat in the promoter region of the gene [A(TA)<sub>7</sub>TAA], is thought to be associated with a reduced SN-38 glucuronidation, causing the higher exposition to SN-38 in patients carrying the polymorphism.

Our pharmacogenetics studies on FOLFIRI regimen indicate that toxicity is lower in patients with TA<sub>6</sub>/TA<sub>6</sub> and TA<sub>6</sub>/TA<sub>7</sub> UGT1A1 genotypes compared to patients carrying the TA<sub>7</sub>/TA<sub>7</sub> genotype.

In this context we proposed a Phase I study to assess the recommended dose of irinotecan according to UGT1A1 genotype for FOLFIRI plus bevacizumab regimen, in patients with mCRC, with the intent of increasing the overall efficiency of the treatment. Eligible patients for this study are genotyped for the UGT1A1\*28 polymorphism and stratified in two groups based on the presence of TA<sub>6</sub>/TA<sub>6</sub> or TA<sub>6</sub>/TA<sub>7</sub> genotypes. Patients with both variant alleles TA<sub>7</sub>/TA<sub>7</sub> are excluded.

This Phase I study is ongoing and therefore we are not able to establish the Maximum Tolerated Dose. Anyway, at the moment, both patient groups demonstrate to tolerate an irinotecan dose higher than the standard one according to the FOLFIRI regimen (180 mg/m<sup>2</sup>) confirming the possibility of personalizing the therapy even in the case of FOLFIRI plus bevacizumab regimen.



## Knock in of cancer mutations in human cells predicts pharmacological response to targeted therapies

Valentina Boscaro,<sup>a</sup> Davide Zecchin,<sup>b</sup> Federica Di Nicolantonio,<sup>b</sup> Alberto Bardelli,<sup>b</sup>  
Margherita Gallicchio<sup>a</sup>

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Recent developments in pharmacogenomics have elucidated the pivotal role of genomic alterations in predicting response to anticancer therapy. We have previously constructed a cell based model system ('knock-in') in which oncogenic point mutations, frequently found in human cancers, have been introduced in a human non transformed epithelial cell line of breast origin (hTERT-HME1). We have inserted the following alleles in hTERT-HME1: EGFR (delE746-A750), KRAS (G13D), BRAF (V600E) and PIK3CA (E545K and H1047R). These 'knock-in' models were utilized to investigate the role of these mutations in tumor progression and to identify genotype- specific pharmacological responses [1]. As tumor suppressor genes, such as PTEN and RB-1, are frequently down regulated in human tumors, we exploited shRNAs to permanently knock-down their expression, both in the parental hTERT-HME1 cells and in the 'knock-in' models. This strategy has allowed combining tumor suppressor gene inactivation with mutational activation of specific oncogenes. The resulting cellular models are hereafter referred to as combinatorial 'matrix'. After biological and biochemical validations, the 'matrix' was used to highlight pharmaco-genetic relationships in the presence of one or multiple cancer mutations. For that purpose, we have selected a panel of drugs routinely utilized in cancer therapy (e.g. erlotinib, trastuzumab, cetuximab, etc.) or compounds in clinical trials targeted against molecular pathways involved in tumorigenesis (e.g., PLX4720, AZD6244, and others) [2]. This library of compounds was screened against the combinatorial cell "matrix" for growth inhibition. The results suggest that specific mutations affecting oncogenes play a key role in dictating the pharmacological response, while down regulation of tumor suppressor genes has a less pronounced effect. These cellular models carrying genetic alterations in both oncogenes and tumor suppressor genes may represent an efficient tool to investigate the role of these alterations in tumor progression and to unveil unknown drug-genotype interactions.

[1] F. Di Nicolantonio et al., *Proc. Natl. Acad. Sci. U S A.* **2008**, *105*, 20864-20869.

[2] W.W. Ma, A.A. Adjei, *CA Cancer J. Clin.* **2009**, *59*, 111-137.



## Pharmacogenomic Markers of Clinical Efficacy in a Dose-Dense Therapy Regimen (R-CHOP14) in Diffuse Large B Cell Lymphoma

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**Background:** Diffuse large B cell lymphoma (DLBCL) is one of the most common types of non-Hodgkin's lymphoma. Approximately half of patients will be cured of their disease by primary therapy, including the R-CHOP regimen (rituximab, doxorubicin, cyclophosphamide, vincristine, desamethasone). The remaining die of the disease, mainly because of the occurrence of tumor drug resistance. Many efforts have been made to explain the biochemical and molecular mechanisms involved in resistance to the drugs used in the treatment of cancer patients, including those with DLBCL. A dose-intense therapy regimen (e.g. R-CHOP14) may help to improve the treatment outcome of DLBCL patients.

**Aims:** We have carried out a retrospective study aimed at correlating the mRNA expression levels of genes involved in metabolism, mechanisms of action and resistance to doxorubicin (i.e. MDR1, GSTP1, TOPO-2 $\alpha$ , Bcl-2, PKC- $\beta$ 2) that represents the backbone of the R-CHOP regimen with treatment outcome data of 54 patients at various stages of disease. **Methods:** The expression of the 5 above mentioned genes was determined in formalin-fixed paraffin-embedded samples from DLBCL using real time RT-PCR. A threshold analysis to identify a cut-off distinguishing recurrent or non-recurrent disease was used. The correlations between gene expression data and clinical/pathological characteristics as well as survival parameters have been evaluated by standard statistical tests.

**Results:** The case series included 32 males and 22 females; 6 patients had follicular lymphoma grade IIIb and 48 diffuse large B cell lymphoma; 19 presented symptoms at diagnosis. Thirty patients showed abnormal LDH values, the IPI was intermediate-high risk or high risk in 14 patients. Forty-six patients (85.2%) obtained a complete remission and 8 (14.8%) a partial response.

The median overall survival (OS) as well as the median progression free survival (PFS) have not yet been reached after a median follow-up of 43.6 months.

The mRNA expression levels of TOPO-2 $\alpha$  and GSTP1 were detectable in all samples, that of PKC- $\beta$ 2 in 52 samples, that of MDR1 and bcl-2 in 34 and 29 samples, respectively. A high degree of interpatient variation in relative tumor expression of the study gene was observed: from 0.008 for TOPO-2 $\alpha$  to >100.000 for PKC $\beta$ II.

Threshold analysis indicated significant inverse relationships between PKC- $\beta$ 2 and PFS (p=0.046): higher gene expression was associated with shorter PFS. Conversely, higher expression of ABCB1 was associated with prolonged PFS (p=0.039). This kind of analysis also showed associations between OS and TOPO-2 $\alpha$ , GSTP1 and PKC- $\beta$ 2: higher gene expression was associated with shorter OS.

**Conclusions:** Overall, our results confirm that the high expression of some genes such as TopoII $\alpha$ , GSTP1 and PKC $\beta$ II may represent a prognostic factor in case of an intensified anthracycline-based chemotherapy with immunotherapy. Moreover, our results suggest that intensified immunochemotherapy could affect the role of bcl2, ABCB1, GSTP1 and TopoII $\alpha$  in predicting tumor response.

These results and others from related studies may help to identify gene profiles useful for selecting patients eligible for more intensified or personalized chemotherapy. Prospective larger studies are warranted.

Supported by a grant from Associazione Giacomo Onlus, Castiglioncello (LI).

## Design and development of a pharmacogenetic molecular test for predicting personalized Warfarin dosages

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An interest for pharmacogenetics in the scientific community has been rapidly growing thanks to the development of methods able to detect mutations and/or SNPs responsible for the resistance to cancer drugs or for side effects to pharmacological therapies [1].

The aim of this study is to design and to develop a method for the analysis of genetic polymorphisms influencing the required Warfarin dose. Warfarin is the most widely prescribed oral anticoagulant for thromboembolic therapy in Europe and North America. However, the risk of thrombotic and bleeding complications is very high especially in the initial stage of Warfarin therapy, since it has a narrow therapeutic window and an inter-individual variability in dose response.[2].

In 2007, the FDA approved the warfarin labeling change, highlighting the opportunity to use genetic tests to improve the initial estimated drug dose. As a result, many algorithms have been developed and tested to predict the individual dose, based on genetic variations and clinical parameters.

By taking into account an Italian algorithm for Warfarin dosage, specific for Caucasian population, four polymorphisms were investigated in this study: rs9923231 in VKORC1 gene, rs1799853 and rs1057910 in CYP2C9 gene and rs2108622 in CYP4F2 gene [3].

The genetic variants were identified using a RLB (*Reverse Line Blot*) hybridization assay that requires a previous step of multiplex amplification. RLB offers some advantages as the simultaneous detection of different mutations and the possible automatization of the protocol, useful for high throughput.

Cytochrome genes have a very similar sequence and contain repeated block sequences that make allelic discrimination and primers/probes construction a challenge. To overcome this issue and to assure amplification specificity, a PCR-SSP (*Sequence Specific Primers*) method was used.

Amplicons were analyzed by direct sequencing and the results showed the specificity of the amplification. Therefore, the multiplex PCR conditions and the hybridization assay protocol were set up on known genotyped samples.

The next step will be to assess the diagnostic specificity and sensitivity of the test.

[1] K.C. Lee, J.D. Ma, G.M. Kuo, *J Am Pharm Assoc*, **2010**, 50(1):e1-14.

[2] N. Roper, B. Storer, R. Bona, M. Fang. *J Mol Diagn*. **2010**, 12(3):283-91

[3] C.F. Zambon, V. Pengo, R. Pedrini, D. Basso, P. Fogar, A. Nisi, A. Frigo, S. Moz, M. Pelloso, M. Plebani, *Pharmacogenomics*. **2011**, 12(1):15-25.



## Pharmacogenomics in the development of new anti-tumor drugs: single-target vs. multi-target

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Genetics is believed to account for between 20% and 95% of the variability in drug disposition and effects. Recently, the field of pharmacogenetics has evolved into “pharmacogenomics,” involving a shift from a focus on individual candidate genes “single-target” approach, to genomewide association studies, “multi-target” approach [1, 2]. A further widening of the area was recently introduced by the pharmacoepigenomics, i.e. the analysis of drug response regulation by epigenetic mechanisms such as tumor associated changes in DNA methylation, histone modification and microRNA expression [3, 4].

Pharmaco-genomics -epigenomics is particularly important in oncology since systemic toxicity and unpredictable efficacy often characterize chemotherapy; furthermore, the high costs of chemotherapy make selection of the appropriate agent relevant for the healthcare systems. As a note of caution, on the basis of the level of evidence required to show clinical utility, the Food and Drug Administration (FDA) has altered drug labels and issued warnings about pharmacogenomic variation affecting drug response [5, 6]. Overall, studies using very large collections of cancer cases, homogeneous well-defined clinical sample sets and adequate validation sets are needed to further characterize the role of polymorphisms or epigenetic changes as putative markers. Owing the possible limitation in patient numbers, the use of functional validation may help verify the results of pharmacogenomic studies.

Clinically relevant examples will be discussed focusing in: pharmacogenetics, mainly involving pharmacokinetic effects (drug metabolism); pharmacogenomics, mainly involving pharmacodynamics effect (drug target); pharmacoepigenomics, mainly involving microRNA profiles.

[1] R.G. Watson, H.L. McLeod, *Cancer J.* **2011**, *17*, 80-88.

[2] L. Wang, H.L. McLeod, R.M. Weinshilboum, *N. Engl. J. Med.* **2011**, *364*, 1144-1153.

[3] A.J. Paige, R. Brown. *Pharmacogenomics* **2008**, *9*, 1825-1834.

[4] M. Toyota, H. Suzuki, T. Yamashita, K. Hirata, K. Imai, T. Tokino, Y. Shinomura. *Cancer Sci.* **2009**, *100*, 787-791

[5] J. Woodcock, *Clin. Pharmacol. Ther.* **2010**, *88*, 765-73.

[6] [www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm](http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm)



## Role of GST-M deletion in azathioprine metabolism in pediatric patients with IBD

E. Cuzzoni<sup>1</sup>, S. De Iudicibus<sup>2</sup>, G. Stocco<sup>1</sup>, R. Franca<sup>3</sup>, F. Bartoli<sup>2</sup>, S. Martellosi<sup>3</sup>, A. Ventura<sup>2</sup>, G. Decorti<sup>1</sup>

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Azathioprine (AZA) is widely used as an immunosuppressive agent in inflammatory bowel disease (IBD). Although its efficacy in maintaining remission is largely accepted, incomplete response is relatively common and adverse drug reactions to this agent occur in 15-38% of patients. AZA is a pro-drug and requires *in vivo* conversion to its active form: the first step in its bio-transformation involves conjugation with sulphide groups and the formation of 6-mercaptopurine (6MP), which has no intrinsic activity but is converted to active monophosphate thionucleotides (TGNs).

Polymorphisms of enzymes involved in the metabolism of AZA influence the efficacy and toxicity of the treatment: in particular thiopurine-S-methyl-transferase (TPMT) variants result in increased production of the active TGN metabolites, however a variable response has been observed also in patient with the wild type TPMT genotype. The conversion of AZA to 6MP is reported as being non enzymatic, nevertheless there is a body of evidence that the enzyme glutathione-S-transferase (GST) is also involved in this conversion. This enzyme presents 7 isoforms among which GST-M is the one most involved in AZA activation. GST-M gene is polymorphic, and the most frequent variant is characterized by a partial deletion, resulting in a complete absence of enzyme activity; the polymorphism occurs with a frequency of approximately 50% in Caucasians.

The aim of this study was to investigate, in pediatric patients with IBD treated with AZA, the correlation between GST-M1 deletion and the concentration of AZA metabolites. 77 pediatric patients with IBD (median age: 15 years, 46 with Crohn's disease, 30 with ulcerative colitis, 1 with indetermined colitis) treated with AZA at least from 3 months, were included in the study. A total of 156 blood peripheral samples were collected (sample per patient: mean=2; range=1-6), and the concentrations of TGN and MMPN metabolites were measured by high performance liquid chromatography (mean±sd TGN: 379±198 pmol/8x10<sup>8</sup> erythrocytes; MMPN: 2008±3142 pmol/8x10<sup>8</sup> erythrocytes). DNA was extracted from peripheral blood and all subjects were genotyped for GST-M polymorphism using PCR-ASO (polymerase chain reaction-allele specific oligonucleotides). Statistical analysis was done considering the median value of metabolites concentration for each patient and the effect of the genotypes was evaluated using Wilcoxon's test.

Of 77 patients considered, 44 had a GST-M wild type genotype, 30 had a null GST-M variant and for 3 patients genotype could not be assessed. GST-M deletion had significant effects on TGN nucleotides: patients with the null GST-M variant showed reduced TGN active metabolites (p=0.033), also considering the ratio TGN/dose (p=0.001). AZA dose was significantly reduced in patient with the null genotype, indeed clinicians adjust the dose on the basis of clinical response (p=0.033).

Further investigation on the role of GST-M deletion are ongoing; in particular the correlation between GST-M genotype and *in vitro* proliferation of peripheral blood mononuclear cells (PBMCs) treated with AZA and 6MP, assessed by [methyl-<sup>3</sup>H] thymidine incorporation.



## Genetic predictors of glucocorticoid response in paediatric patients with inflammatory bowel diseases

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Glucocorticoids (GCs) are used to induce remission in patients with Inflammatory Bowel Diseases (IBD), however a considerable variability in response to these drugs is often evident in paediatric subjects. GCs exert their biological effects through binding to the GC receptor (GR), which regulates either positively or negatively the expression of target genes. Polymorphisms in the GR gene (NR3C1) have been described: in particular the *BcII* polymorphism located in intron 2, was associated with an higher GC sensitivity and has been related with an increased response in patients with IBD. The high inter-individual variation in GC response could depend also on alteration in cytokine production: IL-1 $\beta$  is one of the most important soluble mediators of inflammation and an association between IL-1 $\beta$  production and genotype has been suggested. IL-1 $\beta$  is produced as an inactive cytoplasmatic precursor that must be cleaved to generate the mature active form and NALP1 inflammasome is required for this cleavage. A recent study has shown that variants of NALP1 gene are associated with autoimmune diseases that cluster with vitiligo, and, suggested that mutations in this gene may result in a deregulated secretion of IL-1 $\beta$ , that could therefore alter GC sensitivity. The aim of this study was to identify genetic markers useful to predict the clinical response to steroid treatment in paediatric patients with IBD. 154 young IBD patients, treated with GCs for at least 30 days and with a minimum 1-year follow-up were genotyped. The studied polymorphisms included *BcII* in the NR3C1 gene, C-511T in IL-1 $\beta$  gene, and Leu155His and rs2670660/C in NALP1 gene. Subjects were grouped as GC responders, dependants and resistants. The relation between GC response and polymorphisms was examined using univariate, multivariate and CART (Classification and Regression Tree) analysis. Univariate analysis showed that *BcII* polymorphism was more frequent in responders compared to dependants ( $P=0.03$ ) and to combined steroid dependants and resistants groups ( $P=0.02$ ). Moreover, the NALP1 Leu155His polymorphism was less frequent in the GC responsive group compared to resistant ( $P=0.0059$ ) and non responder ( $P=0.02$ ) groups. Multivariate analysis comparing responders and non responders revealed an association between *BcII* mutated genotype and steroid response ( $P=0.030$ ), and between NALP1 Leu155His mutant variant and non responders ( $P=0.033$ ). An association between steroid response and male gender was also observed ( $P=0.034$ ). In addition Leu155His mutated genotype was associated with steroid resistance ( $P=0.034$ ). CART analyses supported these findings by showing that *BcII* and Leu155His polymorphisms had the greatest effect on steroid response (permutation  $P$  value=0.046).

In conclusion this study has identified two polymorphisms related with GC response in paediatric IBD patients: the *BcII* polymorphism in NR3C1 gene, associated with increased response to therapy, and the Leu155His polymorphism in NALP1 gene related with resistance to treatment. These results confirm that genetic and demographic factors may affect the response to GCs in young patients with IBD and strengthen the importance of studying high order interactions for predicting response.



## Clinical and genetic characterization of dihydropyrimidine dehydrogenase (dpd) deficiency in fluoropyrimidine-treated patients carrying the dpyd\*2a allele

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**Background:** The therapeutic efficacy and toxicity of fluoropyrimidines are, at least in part, related to the balance between anabolism of the drug to its nucleotides, which inhibit thymidylate synthase and are incorporated into RNA and DNA, and the catabolic pathway dependent on dihydropyrimidine dehydrogenase (DPD), which is the initial and rate-limiting step in pyrimidine degradation. Over the last decade, it has become clear that DPD regulates the amount of 5-FU available for anabolism thereby affecting its pharmacokinetics, toxicity, and efficacy. Moreover, an uncommon variant of the DPD gene, consisting of a G to A mutation in the splicing recognition sequence of intron 14 (IVS14+1G>A) of the DPD-encoding gene (DPYD\*2A), produces a non-functional enzyme due to skipping of exon 14 and is potentially associated with life-threatening toxicity. **Aim:** This study provides a description of the clinical features of fluoropyrimidine-induced toxicity in patients homo- or heterozygous for DPYD\*2A. **Patients and methods:** Six patients given FOLFOX, capecitabine or 5-FU test dose (425 mg/mq) were genotyped. They suffered from the following toxicities (WHO criteria): diarrhea and febrile neutropenia grade 3-4, nausea-vomiting, stomatitis, piastrinopenia, alopecia, hand-foot syndrome grade 3 and anemia grade 2. Blood samples for DNA analysis were collected and used to screen patients for DPD polymorphisms by PCR and automatic sequencing of the entire coding region. **Results:** Five patients were found heterozygous IVS14+1GA (DPYD\*1/\*2A) and one patient was homozygous mutant IVS14+1AA (DPYD\*2A/\*2A). The homozygous patient was initially tested with a reduced 5-FU test dose and showed diarrhea grade 2, mucositis grade 3, anemia grade 1, piastrinopenia grade 3, febrile neutropenia grade 4, complete alopecia and *Staphylococcus aureus* sepsis. This patient required 20 days of hospitalization and was managed with antibiotics, platelet transfusion, port removal, G-CSF administration and parenteral nutrition. **Conclusions:** Although the frequency of DPYD\*2A allele is low, the screening for DPD mutation is clinically relevant to avoid the severe toxicities or death in patients treated with fluoropyrimidine-containing regimens.



## Targeted therapy, proof of principle and biomarkers

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Cancers having a similar histotype, stage, and grade of the disease, can respond differently to the same therapy. The explanation for this therapeutic failure can be partly ascribed to the complexity and heterogeneity of tumor genetics.

Cancer cells are often dependent upon the continued activity of mutated oncogenes for maintenance of their malignant phenotype even in the presence of additional tumorigenic lesions. The dependence from an activated signalling or mutated oncogene in cancer is referred to as 'oncogene addiction' (1). Therefore, addiction to cancer genes represents chinks in the tumour's armour and can be therapeutically exploited. However, new cancer drugs 'targeted' to inhibit oncogenic signalling pathways have shown modest results in clinical trials. Indeed, in most cases, the response to these drugs involves only a subset of patients, suggesting that the presence of a complex of molecular alterations is responsible for different biological behaviour of the cancer cells and for the ultimate clinical outcome.

As an example, we and others have shown that EGFR targeted therapies in metastatic colorectal cancer (mCRC) are effective in only 10-20% of unselected cases. It is now well documented that the presence of specific oncogenic alterations in RAS genes that are downstream the EGFR signalling cascade could by-pass the effect of EGFR targeted agents in this setting. Indeed, the European Medicine Agency (EMA) and the FDA have approved the clinical use of cetuximab or panitumumab (monoclonal antibodies targeting EGFR) for mCRC patients whose tumors carry wild-type KRAS. The occurrence of KRAS mutations, however, only accounts for about 30-40% of non-responsive mCRC patients. This percentage can be increased to over 70% when we consider the subgroup of patients affected by tumours that do not present genetic alterations downstream the EGFR signalling pathway (cancers that are wild-type for KRAS, NRAS, BRAF, PIK3CA and PTEN).

The development of validated predictive molecular markers will not only spare patients ineffective and toxic therapies, but will also greatly reduce futile costs. In fact, it has been estimated that in the US the exclusion of patients with KRAS mutations from treatment with EGFR-targeted therapies could result in over \$700 net million savings, after considering the cost of the simple KRAS test. As novel targeted drugs progress from advanced to first-line treatment, it is expected that the discovery and validation biomarkers of response/resistance will accelerate, resulting in their implementation in early clinical trials, with improved patient care and a more rational use of healthcare resources.

[1] Weinstein, *Science*, **2002**, 297, 63.



## **microRNA and pharmacogenetics: focus on neoadjuvant treatment for rectal cancer**

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**BACKGROUND:** Over the past decade, a lot of studies have been conducted to better understand the function of microRNAs, a new class of 20-24nt non-coding RNAs that regulate gene expression primarily through post-transcriptional repression or mRNA degradation in a sequence-specific manner. They play an important role in various cellular pathways. Increasing evidences show that polymorphisms affecting microRNAs activity can be used as new potential biomarkers in the oncologic field.

**AIM:** In this study we have focused our attention on the identification of polymorphisms affecting miRNA pathways that could be used as potential predictive biomarkers for the tumor response to neo-adjuvant chemo-radiotherapeutic treatment in rectal cancer patients in term of Tumor Regression Grade (TRG).

**METHODS:** To select candidate polymorphisms in miRNA related genes, we have conceived a research strategy based on the use of different bioinformatics algorithms and literature analysis. We also included in the analysis polymorphisms in pathways known to be involved in the response to radiotherapy, as DNA repair, angiogenesis and EGFR pathways. 21 polymorphisms in 14 different genes were analyzed in 122 patients with locally advanced rectal cancer treated with neoadjuvant radio-chemotherapy 5-fluoruracil-based. Genomic DNA was extracted from peripheral whole blood. Genotyping was performed by Pyrosequencing, allelic discrimination by TaqMan technology, automated fragment analysis. Fisher's Exact test was applied for data analysis.

**RESULTS:** TRG was significantly predicted by a polymorphism in a gene encoding for a miRNA: miR100 (rs1834306). A predictive role was also associated to 2 polymorphisms related to DNA repair pathways: Tp53 IVS2 38C>G (rs1642785) and hMSH6 556G>T (rs3136228). Patients homozygous for the variant allele of a polymorphism of miR100 (rs1834306) and of Tp53 IVS2 38C>G (rs1642785) showed a worse response to the treatment (TRG $\geq$ 4) (OR=0.344, CI=0.132-0.898, P-value=0.034; OR=0.147, CI=0.027-0.785, P-value=0.020 respectively), while patients heterozygous for hMSH6 556G>T (rs3136228) responded better (TRG $\leq$ 2) to the therapy (OR=4.571, CI=1.275-16.388, P-value=0.022). These results will be confirmed in a validation set of patients.

**CONCLUSION:** This work shows the noteworthy potentiality of studying the miRNA pharmacogenetics in cancer patients. Three exploratory genetic biomarkers of tumor response in locally advanced rectal cancer, treated with neoadjuvant radio-chemotherapy have been highlighted. If confirmed in further studies, they could be useful for better treatment personalization.



## **Pharmacogenetic of warfarin and its applications in the community medicine**

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Warfarin is widely used for oral anticoagulant therapy, and a careful monitoring of therapy is required, because of the narrow therapeutic window and high inter-individual variability in the dose needed which exposes the patients to a relevant risk of thrombotic or bleeding complications. Anticoagulative response to warfarin is influenced by a number of clinical and genetic factors. Single nucleotide polymorphisms in genes affecting warfarin metabolism (CYP2C9) and pharmacodynamic response (VKORC1) have been strongly associated with warfarin responsiveness [1]. 85 patients from the Centre for Cardiovascular Disease of the local Azienda per i Servizi Sanitari n.1 Triestina, have been recruited and genotyped. The analysis of the variance (ANOVA) of the data confirms the role of VKORC1 and CYP2C9 in determining the warfarin dose required (df=2; F=8.175; p=0.001 and df=4; F=4.254; p=0.004). Linear regression analysis, conducted to construct a model and to evaluate the weight of the clinical and genetic characteristics influencing dosing, indicates the significant factors are the genetic polymorphisms considered together with the smoking status of the patients, which was positive in 5 cases. The enrollment and analysis of more patients continues, including selected subgroups with a high variability in the optimal dosage as identified by the routine use of INR, with the aim to evaluate whether genetic factors are responsible for events requiring medical care during anticoagulation, in addition to their role for the choice of initial dosage.

The results presently obtained indicate encourage the application of genotyping also for warfarin anticoagulant therapy in community care. They also encourage the examination of the possible associations of the genetic polymorphisms affecting warfarin pharmacology with haemorrhagic events and the abuse of recreational drugs.

[1] The International Warfarin Pharmacogenetics Consortium (2009) N Engl J Med 360 (8):753



## **Pharmacogenomics and the Pediatric Cancer Genome Project**

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The Pediatric Cancer Genome Project was launched in 2010 as a collaboration between St. Jude Children's Research Hospital in Memphis, TN, USA and the Genome Center at Washington University in St. Louis, MO, USA, to sequence and compare the complete normal and cancer genomes of 600 children and adolescents with leukemia, solid tumors or brain tumors. It is anticipated that the three-year effort will revolutionize understanding the genomics of childhood malignancies and lay the foundation for new treatments to cure or prevent cancer, which remains the leading cause of death by disease in US children over one year of age. The genome project takes advantage of next-generation sequencing technology, coupled with evolving informatics tools and extensive integration with biological and clinical data at St. Jude. Perspectives on the relevance of this project for pharmacogenomics and personalization of therapy for children with cancer will be provided.



## Childhood Acute Lymphoblastic Leukemia (ALL): role of polymorphisms in the GST- $\mu$ , GST- $\theta$ and ABCC1 genes on relapse

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In the AIEOP-BFM-ALL 2000 trial (Associazione Italiana Ematologia Oncologia Pediatrica/ Berlin-Frankfurt-Münster Acute Lymphoblastic Leukaemia Study Group), 15% of paediatric patients treated according to risk-adapted poly-chemotherapeutic regimens relapsed, suggesting the need of criteria additional to those based mainly on the minimal residual disease to better predict an adverse clinical outcome. The aim of the present study was to investigate whether the common homozygous deletion ("null genotype") of glutathione S-transferase- $\mu$  (GST-M1) and - $\theta$  (GST-T1) as well as single nucleotide polymorphisms (SNPs) of other genes (among which ABCC1 SNP rs3784862) could become such novel prognostic genetic markers. We proposed an innovative statistical approach (two-phase design) in order to select a sub-sample of ALL patients to be genotyped from the whole cohort of 1999 patients treated with the AIEOP-BFM-ALL 2000 protocols by the use of the clinical information available, with a considerable gain in efficiency. A total of 614 patients was genotyped by multiplex polymerase chain reaction (PCR) and TaqMan<sup>®</sup> technology; relapse cumulative incidence was estimated by weighted Kaplan-Meier, and Cox model for two-phase design was applied to evaluate the genotypes effect on relapse in the whole Italian cohort. Overall analysis did not show any significant result. However, when patients were stratified by risk group, age class or *in vivo* prednisone pre-phase response, the prognostic significance of gene variants towards an higher rate of relapse emerged for GST-M1 normal, GST-T1 null and mutated ABCC1 SNP rs3784862 subjects in specific patient subsets (*GST-M1 normal*:  $p=0.052$ , HR=0.54, 95%CI: 0.29-1.00 in adolescents (>10 years old) and  $p=0.026$ , HR=0.45, 95%CI: 0.23-0.91 within prednisolone poor responders (PPR); *GST-T1 null*:  $p=0.045$ , HR=2.48, 95%CI: 1.02-6.01 in 6-9 years old patients,  $p=0.045$ , HR=4.62, 95%CI: 1.04-20.6 in the standard risk group and  $p=0.041$ , HR=1.62, 95%CI: 1.02-2.58 within prednisolone good responders (PGR); *mutated ABCC1 SNP rs3784862*:  $p=0.03$ , HR=3.38, 95%CI: 1.16-9.86 in adolescents). Although ABCC1 SNP rs3784862 and GST- $\mu$  or - $\theta$  gene variants were independent on their effect on relapse (interaction term  $p=0.17$  and  $p=0.085$ , respectively), analysis of specific contrasts in combined genotypes showed that the adverse role of the mutated ABCC1 emerged clearly within both the normal GST-M1 ( $p=0.021$ , HR=2.31, 95%CI: 1.13-4.69) and GST-T1 patients ( $p=0.009$ , HR=2.21, 95%CI: 1.22-3.99). Our results suggest the potential clinical use of GST-M1 and GST-T1 genotyping as additional prognostic genetic markers of relapse for high (PPR) and standard risk patients respectively, whose risk has been defined according to the already-in-use assessment criteria. GST-M1 and ABCC1 SNP rs3784862 could play an important predictor role for paediatric patients older than 10 years, independently from the risk classes they are belonging.



## **A genotype-guided phase I study for weekly paclitaxel in ovarian cancer patients**

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Paclitaxel is a broad spectrum anticancer agent belonging to the taxane family thus exerting its cytotoxic effect stabilizing the cell microtubules. It is used either as single agent or in multidrug regimens of chemotherapy for ovary, breast, head and neck, prostate and non-small cell lung cancers.

Paclitaxel is metabolized primarily in the liver and is also a substrate for P-glycoprotein (P-gp), an ATP-dependent drug export pump encoded by the gene ABCB1 (MDR1). P-gp is responsible for the reduction in the intracellular accumulation of a wide range of compounds, included cytotoxic drugs. Although P-gp is expressed in normal tissues, high expression of the protein on tumour cells leads to chemoresistance and appears to be correlated to poor response to treatment.

The large inter-individual variability in therapeutic effect and in severity of toxicity is a clinically relevant problem in paclitaxel treatment and could be related to the genetic characteristics of patients, such as Single Nucleotide Polymorphisms (SNP).

Previous studies pointed out the effect of one of the most frequent SNP of the ABCB1 gene in caucasian population, G2677G>T/A, on P-gp protein expression and also a correlation with drug clearance: a reduced P-gp mediated transport from blood to intestine would affect paclitaxel pharmacokinetics, increasing systemic drug exposure.

In a pilot study, carboplatin/paclitaxel –treated ovarian cancer patients carrying the variant 2677T/A allele had lowered paclitaxel clearance (unpublished data).

We planned a dose-escalation phase I study to assess the recommended dose for weekly paclitaxel monotherapy according to ABCB1-2677G>T/A genotype in epithelial ovarian cancer patients, from the starting dose of 80mg/m<sup>2</sup>.

Genomic DNA is extracted from whole blood fractions using the High Pure PCR Template preparation Kit; ABCB1-2677G>T/A promoter polymorphism is analyzed by Pyrosequencing.

Additional polymorphisms of ABCB1 and other genes encoding for key transporters and metabolizing enzymes are also monitored by Pyrosequencing and Taqman assay.

The pharmacokinetic profile of paclitaxel is evaluated during the first chemotherapy cycle, on the first and on the fourth administration, because of the metabolic autoinduction effect of this taxane. Paclitaxel and its main metabolite, 6 $\alpha$ -hydroxypaclitaxel, will be quantified in plasma sample using a new LC-MS/MS method specifically developed.



## Role of EZH2 SNPs in metastatic Colorectal Cancer patients treated with FOLFIRI regimen

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Metastatic colorectal cancer (mCRC) is one of the leading causes of cancer death in Western countries (1). For the majority of mCRC patients, doublet chemotherapy with a fluoropyrimidine plus either irinotecan or oxaliplatin combined with a biologic agent is considered the preferred treatment option in first-line (2). In particular bevacizumab, a monoclonal humanized antibody directed against the vascular endothelial growth factor (VEGF), has been shown to extend overall survival in patients treated with 5-fluorouracil-based chemotherapy (3), and is approved in combination with fluoropyrimidine-based chemotherapy for the first- or second-line treatment of mCRC patients. Cancer stem cells (CSCs) were recently shown to drive colorectal cancer (CRC) progression and chemoresistance (4). Polycomb group genes (PcGs) are epigenetic modifiers involved in CSCs self-renewal through oncosuppressor gene silencing (5). EZH2 is a PcG member, that mediates gene silencing through histone-H3 lysine-27 methylation (5). Recently, 4 *EZH2* single nucleotide polymorphisms (SNPs) have been characterized three of them are associated to lung cancer risk (rs: 3757441, 41277434, 6950683) and one (rs2302427) is responsible for a coding sequence change. The aim of this study was to evaluate the correlation between *EZH2* SNPs and outcome in mCRC patients and their prognostic and predictive role in mCRC patients. DNA was extracted from blood samples of 110 mCRC patients treated with first-line FOLFIRI plus bevacizumab. Genotyping was performed by Real-TimePCR. Oncomine meta-analysis on microarray data showed that a set of *EZH2* target genes are specifically silenced in FOLFIRI-non responder CRC patients. One allelic variant (rs3757441 C/C vs. C/T or T/T) was significantly associated to shorter Progression free survival (PFS) (8.7 vs 11 months, respectively; HR=0.2689; p=0.0076) and Overall survival (OS) (23.8 vs 18.3 months; HR=0.329; p=0.049). At multivariate analysis, the same variant resulted an independent predictor of both PFS and OS (p<0.05) Through analysis by dedicated softwares we investigated the functional relevance of the rs3757441 SNP, finding that it can affect binding to XBP transcription inhibitor. Among 50 patients analysed for *EZH2* expression and genotyped for *EZH2* rs3757441 SNP, mRNA levels were significantly higher in patients harbouring the C/C genotype with respect to C/T and T/T (p<0.05). To confirm these results, we studied the rs3757441 SNP in a parallel set of 104 mCRC patients, treated with FOLFIRI regimen. The C/C genotype was still associated to shorter PFS and OS (p<0.05, log-rank test). Our results indicate that the rs3757441 SNP is associated to higher *EZH2* expression and may be useful to predict PFS and OS in mCRC patients treated with FOLFIRI regimen.

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## Platinum derivate toxicity and lung cancer: The predictive role of genetic polymorphisms.

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**INTRODUCTION:** Lung and pleural cancers are the solid tumors with the highest frequency of mortality worldwide. The severity of this pathological condition is determined by different factors among which relevant are the lack of appropriate and efficient predictive screening tests and the poor response to common antineoplastic pharmacological treatments (PT). According to the EMA guidelines, the recommended PT is essentially a combination of platinum compounds (PC) (cis-platin or carbo-platin) associated with a 3<sup>rd</sup> generation chemotherapeutic drugs such as docetaxel or gemcytabin. The objective of this pharmacogenetic study was to identify potential predictive markers able to provide additional information to physicians both in terms of diagnosis and personalized therapeutic approaches.

**METHODS:** 63 patients with a diagnosis of lung cancer or mesothelioma treated with a platinum derivate were enrolled. The study was conducted enrolling 63 patients, with a consent signed form, diagnosed with lung cancer, all treated with platinum derivatives. The genotype was analyzed for five different SNPs known to play key role in the PC pharmacokinetics/pharmacodynamics, namely GSTP1 313A>G (rs1695), XRCC1 28152G>A (rs25487), ERCC1 8092C>A (rs3212986), ERCC1 19007T>C (rs11615). Major baseline patients characteristic and therapy outcome such as age at diagnosis, smoke, cancer histological staging, renal and hematological toxicity were also recorded. Statistical analysis was performed using the ANOVA and MANOVA tests. A Kaplan Meier curve and a log rank test were also performed to evaluate possible links between SNPs and the overall patient survival.

**RESULTS:** 78% of enrolled patients were diagnosed with non-small cell lung cancer (NSCLC), 13% with small cell lung cancer (SCLC) and 9% with pleuric mesothelioma. High grade hematological (HGET) and non-HGET (mainly gastrointestinal and renal) adverse drug reactions (ADR) were reported in 29% and 39% of patients, respectively. Partial or complete recovery was observed in 48 % of the patients while 31% and 21% were stable or unresponsive to the treatment, respectively. In terms of correlation between the outcome variables treatment and patients' genotypes, toxic effects of PT, HGET correlated with ABCB1 3435C>T (rs1045642) after 3<sup>rd</sup> cycle (p=0.001) and considering the cumulative doses (p=0.002). Gastro enteric toxicity was linked to ABCB1 3435C>T (rs1045642) (p=0.043). GSTP1 313A>G (rs1695) was associated with higher scores in the ECOG-PS scale after 1<sup>st</sup> cycle (p=0.01). GSTP1 313A>G (rs1695) SNP correlated with a better survival (p=0.024); ERCC1 8092C>A (rs3212986) and ERCC1 19007T>C (rs11615) correlated with longer survival time (p=0.003 and p=0.008).

**CONCLUSIONS:** The results obtained indicated a possible association between SNPs and clinical and therapeutic outcomes in lung and pleural cancer. The study also suggests that pharmacogenetic approaches can be used to identify predictive and /or prognostic markers for ADR development in NSCLC, SCLC and pleural mesothelioma under PT treatment.



## Influence of constitutional polymorphisms on response to 5-FU-based chemotherapy in colorectal cancer patients: preliminary results

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**Background.** Response to anticancer agents may be strongly affected by interpatient genetic variability. Thymidylate synthase and methyltetrahydrofolate reductase (MTHFR) are key enzymes in the mechanism of action of 5-fluorouracil (5-FU), the first being the target of the drug and the second determining the intracellular concentration of reduced folates whose availability is a required condition for the 5-FU activity. Three polymorphisms of *TYMS* and two of *MTHFR* have been proposed to have a role in the clinical response (efficacy and toxicity) to 5-FU.

*TYMS* polymorphisms are a tandemly repeated sequence (2R/3R) in the 5'UTR, a SNP within the 3R allele and a 6 bp deletion in the 3'UTR. *MTHFR* polymorphisms are two SNPs: C677T and A1298C.

**Aims.** To evaluate the influence of *TYMS* and *MTHFR* polymorphisms on the efficacy and toxicity of 5-FU-based chemotherapy in colorectal cancer (CRC) patients at various stages of disease.

**Methods:** Within a retrospective and prospective pharmacogenetic study, we have analysed so far the *TYMS* tandemly repeated sequence (2R/3R) and the C/G SNP in the 5'UTR, the 6bp deletion in the 3'UTR and the *MTHFR* C677T and A1298C SNPs in peripheral blood from 37 CRC patients who underwent adjuvant chemotherapy and from 20 CRC patients who underwent first line chemotherapy. Relationships between genotypes and outcome of chemotherapy were investigated.

**Results.** No correlations between genotypes and clinical/pathological characteristics or efficacy data (survival parameters and objective response) were not observed in either adjuvant or advanced setting.

In the adjuvant setting, a higher incidence of diarrhea was observed in patients carrying at least a *MTHFR* 677T allele compared to *MTHFR* 677CC patients ( $p=0.047$ ). A trend between overall toxicity and *TYMS* 2R/3R polymorphism was also observed: patients with wild-type genotype had a higher incidence of severe toxicity compared to patients carrying at least a rare allele ( $p=0.073$ ).

Hematological toxicity was found to be associated with the 2R/3R polymorphism in advanced disease: heterozygous genotype patients showed higher incidence of anemia compared to either homozygous mutant and wild type patients ( $p=0.035$ ). Finally, a lower incidence of anemia was observed in heterozygous *MTHFR* A1298C patients ( $p=0.034$ ).

**Conclusions.** Although controversial results are reported in the literature on the role of the above reported gene polymorphisms in the outcome of CRC patients, our preliminary data are in agreement with most of the available studies which showed no involvement of *TYMS* or *MTHFR* gene polymorphisms in 5-FU efficacy but did suggest its involvement in toxicity. However, the higher number of patients that will be reached at the end of our pharmacogenetic study, will allow us to confirm our findings.



## Restoration of Raf-1 Kinase Inhibitor Protein levels as a possible therapeutic approach in hepatocellular carcinoma

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Raf-1 Kinase Inhibitor Protein is a tumor and metastasis suppressor and is down-regulated in several cancer types. It inhibits the oncogenic activities of MAPK and NF- $\kappa$ B pathways and promotes drug induced apoptosis. In particular, we and others have shown highly consistent reduced RKIP expression, both at mRNA and protein level, in human hepatocellular carcinomas (HCCs) compared to adjacent peritumoral cirrhotic tissues or to healthy livers [1,2]. Interestingly, the mechanism of RKIP coincides, at least in part, with that of Sorafenib, which at present is the only drug approved for the treatment of advanced HCC. Overall, the possibility of restoring RKIP might represent a new important intervention for the therapy of HCC. For this reason we are investigating about the mechanism(s) of RKIP down-regulation, of which little is known, especially in the case of HCC. We have sequenced the promoter and coding regions, including the exon/intron boundaries, of the *RKIP* gene in human HCC cell lines (HA22T/VGH, HepG2, Hep3B) and in five clinical HCC samples and, even if we have found many genetic variants, we have ruled out that DNA mutations can be responsible for the lowered RKIP expression.

It is known that epigenetic changes, like histone deacetylation or DNA methylation, can alter gene expression. In the HA22T/VGH and HepG2 cells, the histone deacetylase inhibitor trichostatin (TSA) induced antiproliferative and apoptotic effects and exhibited antitumor synergy with doxorubicin. TSA caused also histone H3 hyper-acetylation and modified the expression of different relevant genes (like  $\beta$ -catenin, cyclin D1, hTERT, XIAP and IL-6). Nevertheless, TSA did not modify the low basal levels of RKIP mRNA and protein in the two HCC cell lines.

Further, we have analyzed *RKIP* gene promoter methylation in the HCC cell lines and in the five tumor samples; only the Hep3B cells showed a moderate methylation of the gene, which was reverted by treatment with the demethylating agent 5-aza-2'-deoxycytidine. The same treatment caused also up-regulation of RKIP at mRNA but not at protein level. In the tumor samples, the gene was not found to be significantly methylated.

It has been shown that in prostate cancer RKIP expression is negatively regulated by SNAIL, a transcriptional repressor activated by NF- $\kappa$ B [3]. However, we could not find any relationship between the expression of SNAIL and the levels of RKIP mRNA in the HCC cell lines and in the clinical tumor samples.

Finally, up-regulation of microRNAs (miRNAs) has been associated to cancer progression, due to inhibition of tumor suppressor genes expression. Our preliminary data have shown up-regulation of different miRNAs (miR-1, miR-7, miR-18a, miR-21, miR-34b, miR-221, miR-224) in clinical HCC samples; according to computational analysis, RKIP is one of the targets of miR-224, which thus might cause RKIP mRNA reduction.

Overall, RKIP may be down-regulated both by transcriptional and post-transcriptional mechanisms in human HCCs. Further studies are necessary to better identify these mechanisms, also in order to find therapeutic approaches able to overcome RKIP dysregulation in this tumor.

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## Association Between Genotypes and Response to the treatment in a Subset of Previously Untreated Chronic Myeloid Leukemia Patients

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Imatinib mesylate (IM) is the gold standard for chronic myeloid leukemia (CML) treatment. Some patients however, experience suboptimal response or resistance which highlights the possibility for a further treatment optimization, potentially guided by molecular predictors of response. Since blood and tissue concentrations of drugs may be influenced by interindividual variations, like SNPs, in genes encoding drug metabolizing enzymes and drug transporters, we have genotyped a panel of SNPs in genes known to transport IM, in a subgroup of 189 patients enrolled into TOPS (Cortes, JCO, 2010). Each SNP was assessed for associations with cytogenetic response (CgR) and molecular response (MR). We found that MDR1 rs1045642 SNP was statistically significantly associated with CgR achieved within 12 months ( $p=0.044$ ). We also tested associations with CgR or MR and MDR1 and hOCT1 SNPs at the haplotype level. For both MDR1 and hOCT1, correlations of patients status and CgR achieved within 12 months had  $p$ -values between 0.064 and 0.18. Potentially, if the same level of association were seen in a larger sample size the results could reach statistical significance. In conclusion, in this preliminary exploratory study we found that genotypic variations in IM transporters may be associated with response to IM. Our plan is to conduct the analysis on a larger series of patients to further expand these observations, and to better investigate potential correlations with treatment outcome, treatment regimen and drug levels.

This investigation was conducted by CML Correlative Studies Network (CCSN), TOPS, which is sponsored by Novartis Oncology.



## 5-HTTLPR Polymorphism and mental adaptation to cancer

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Difficulties in coping with cancer, and the accompanying anxious and depressive symptoms, have been shown to affect the mood and the quality of life in breast cancer patients. 5-HTTLPR (5-Hydroxytryptamine Transporter Gene-linked Polymorphic Region) functional polymorphism of serotonin transporter has been shown to influence the adaptation to stressful life events. The aim of this study was therefore to examine the association of 5-HTTLPR with the mental adaptation to cancer diagnosis and treatment.

48 consecutive patients with early mammary carcinoma were evaluated at enrolment, and at follow up after one and three months. The patients were characterized psychometrically using the Hospital Anxiety and Depression Scale and the Mini-Mental Adjustment to Cancer Scale (Mini-MAC); 5-HTTLPR allelic variants were determined using conventional techniques. The mental adaptation to the disease was associated with high scores of avoidance and anxious preoccupation of Mini-MAC, which decreased with time at follow up. Anxious preoccupation decreased with time less in patients with the S/S and S/L genetic variant of 5-HTTLPR as compared with the LL carriers ( $p=0.023$ ), indicating a Gene X Environment interaction.

In patients with early or advanced tumors, the scores of hopelessness-helplessness (HH) and anxious preoccupation (AP) of Mini-MAC significantly correlate with those of depression and anxiety of HADS; the correlation of HH with HADS depression significantly depend on 5-HTTLPR L/L variant only in patients with early breast cancer.

Pharmacogenetic data on SSRIs antidepressant drugs are available, indicating that their effects are more pronounced in depressed patients carrying the L/L 5-HTTLPR polymorphism. Results in accord with these findings have been obtained in the Hospice were patients with different advanced tumors which were carriers of the L/L genotypic variant displayed a significantly higher response to sertraline, citalopram and escitalopram in terms of reduction of depressive symptoms as well as amelioration of the mental adjustment to the disease.

The results reported indicate that the characterization of 5-HTTLPR may allow the identification of breast cancer patients in greater risk of mental suffering, for which specific intervention may be focused; in the case of drug therapy, they provide indications which may be used for the choice of most appropriate agent in a pharmacogenetic perspective.



## Genome-wide identification of determinants of leukemia cell sensitivity to mercaptopurine

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The sensitivity of primary leukemia cells to anti-leukemic medications at diagnosis, as measured with the in vitro drug cytotoxicity assay MTT, has prognostic value in childhood acute lymphoblastic leukemia (ALL) and may therefore, be used to elucidate the mechanisms of de novo drug resistance [1]. Previous studies have shown that in vitro resistance to prednisone, vincristine and asparaginase is significantly associated with treatment response (remission induction failure or relapse within 2.5 years of diagnosis) [2]. Differential expression of a relatively small number of genes, identified by high-throughput agnostic microarray analysis, is significantly associated with de novo drug resistance (MTT assay) and treatment outcome in childhood ALL [1]. These studies have provided new insights in the pharmacodynamics of anti-leukemic medications, for example identifying the contribution of members of the SWI/SNF transcription complex, such as SMARCB1, as associated with glucocorticoid resistance in ALL cells [3]. High-throughput gene expression studies have not yet been performed to assess ALL resistance to the thiopurine antimetabolite 6-mercaptopurine (6MP). Therefore an analysis was done to identify sets of differentially expressed genes (mRNAs) in primary ALL cells that were sensitive versus resistant to 6MP. At St. Jude Children's Research Hospital in Memphis TN (USA) and at Erasmus Medical Center (EMC) in Rotterdam (Netherlands) we tested primary leukemia cells from newly diagnosed children for in vitro sensitivity to 6MP; the cells were then subjected to an assessment of gene expression with the use of 22,277 probe sets to identify differentially expressed genes in drug sensitive and drug resistant ALL. Complete data was obtained for 121 patients at St. Jude (104 B-lineage and 17 T-ALL) and 156 patients at EMC (127 B-lineage, 29 T-lineage). ALL cell gene expression analysis was done by comparing gene expression in the most sensitive vs the most resistant quartiles of the population, using t-test or in the whole population using Spearman's rank correlation. We identified sets of differentially expressed genes in ALL cells that were sensitive or resistant to 6MP in the St. Jude or EMC cohorts. Application of gene expression signatures identified in one cohort and random forest models to estimate sensitivity and resistance to 6MP in the other cohort showed a significant correlation between the estimated values and the measured values. The gene signatures identified contained, among others, genes plausibly related to 6MP induced cytotoxicity, such as HMGB1 and ADA. These results indicate that global patterns of gene expression are related to 6MP sensitivity in ALL blasts at diagnosis. Mechanistic validation of candidate genes is being performed using appropriate experimental techniques (e.g. gene knockdown), in order to elucidate the molecular mechanism(s) connecting thiopurine sensitivity to genes identified. This characterization of 6MP pharmacodynamics in ALL through pharmacogenomics could provide clinically translatable insights to optimize the therapy of pediatric ALL, in order to increase treatment efficacy and to reduce the adverse events of chemotherapy.

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## Association of common variants of GSTP1, GSTA1 and TGFβ1 genes with the risk of radiation-induced *subcutaneous* fibrosis in breast cancer patients

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Radiotherapy is commonly applied after breast-conserving surgery to reduce the risk of loco-regional recurrence of breast cancer, however patients vary considerably in their normal tissue responses to radiotherapy. In order to provide new insights on the genetic basis of normal tissue radiosensitivity, in the present study we evaluated the association between eight polymorphic variants located in six genes related to DNA repair mechanisms, oxidative stress and fibroblast proliferation (XRCC1 Arg399Gln, XRCC1 Arg194Trp, TP53 Arg72Pro, GSTP1 Ile105Val, GSTA1 C-69T, eNOS G894T, TGFβ1 C-509T, TGFβ1 T869C) and the risk of subcutaneous fibrosis in a retrospective series of patients who received radiotherapy after breast conserving surgery.

Subcutaneous fibrosis were scored according to the LENT-SOMA scale in 257 breast cancer patients who underwent surgery plus adjuvant radiotherapy. Genotyping was conducted by PCR-RFLP analysis on genomic DNA extracted from peripheral blood. The association between genetic variants and the risk of moderate to severe fibrosis was evaluated by binary logistic regression analysis.

Two hundred thirty-seven patients were available for the analysis. Among these, 41 patients (17.3%) developed moderate to severe fibrosis (G2-3) while 196 (82.7%) patients displayed no or minimal fibrotic reactions (G0-1). After adjustment of confounding factors (age, BMI, breast diameter, follow-up, smoking status, history of vasculopathy, adjuvant treatment, dose per fraction, radiation quality and boost method) GSTP1 Ile105Val (OR: 2.756, 95%CI: 1.188-6.393, P=0.018), GSTA1 C-69T (OR: 3.223, 95%CI: 1.176-8.826, P=0.022) and TGFβ1 T869C (OR: 0.295, 95%CI: 0.090-0.964, P=0.043) polymorphisms were found to be significantly associated with the risk of G2-3 radiation-induced fibrosis. In the combined analysis, carriers of 3 risk genotypes were found to be at higher odds to develop G2-3 fibrosis compared to patients with 2 risk genotypes (OR: 4.415, 95% CI: 1.553-12.551, P= 0.005) or 0-1 risk genotype (OR: 8.563, 95% CI: 2.671-27.447, P=0.0003).

These results suggest that functional variations in genes involved in oxidative stress response and fibroblast proliferation may modulate the development of radiation-induced fibrosis in breast cancer patients. Results of the combined analysis support the notion that normal tissue radiosensitivity is a polygenic trait dependent on the combined effect of several polymorphic genes and suggest that approaches based on multiple genetic markers could have the potential to predict normal tissues responses after radiotherapy.





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