of *MPL* mRNA in NCI-H510 cells did not correlate with detectable TpoR protein expression. Erythropoietin receptor (*EPOR*) mRNA was expressed at low-to-moderate levels, while *ERBB2* and *IGF1R* were expressed at higher levels. Microarray analysis showed undetectable *MPL* mRNA levels in all breast cancer and RCC samples and low levels in 48% NSCLC samples. In contrast, *EPOR* was expressed in 75–100% of the breast cancer, NSCLC, and RCC samples. *ERBB2* was expressed in 81–100% of the samples and *IGF1R* was expressed in 54–100% of the samples. For breast tumors, the levels of mRNA expression were as follows: *MPL* < *EPOR* < *IGF1R* < *ERBB2* < *epor* < *igf1r* < *erbb2* </>. By qRT-PCR, there were also low or undetectable levels of *MPL* expression in these samples from subjects with prostate, ovarian, lung, and breast tumors. *EPOR*, *ERBB2*, and *IGF1R* expression vary according to tumor type, but were generally higher than *MPL*.
**Conclusions:** In summary, low or undetectable levels of *MPL* mRNA expression were observed in most tumor cell lines and in most samples of patient tumors, compared with *EPOR*, *ERBB2*, and *IGF1R*.

**1094** POSTER

**Influence of TGFB1+869T>c polymorphism in non-small cell lung cancer (NSCLC) risk**

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**Background:** Lung cancer (LC) is the third most common type of cancer in Europe and was the first cause of death by cancer in 2006. Non-small cell lung cancer (NSCLC) accounts for 75%–85% of all histological types of lung cancer. Lung carcinogenesis is complex and a multi-step process, resulting from exposure to environmental and genetic factors. The transforming growth factor beta 1 (TGFβ1) is a multifunctional regulatory polypeptide that controls many aspects of cellular function (cellular proliferation, differentiation, migration, apoptosis, immune surveillance). Nevertheless, TGFβ1 has been suggested to play a dual role, acting as a suppressor in early stages and as a tumor promoter in later stages. *TGFB1+869T>C*, is a functional polymorphism described in *TGFB1* gene responsible for a T-to-C substitution at nucleotide 29 of codon 10. This transition has been associated with higher circulating levels of TGFβ1, that may influence LC development and prognosis. Our purpose was to investigate the role of *TGFB1+869T>C* functional polymorphism in NSCLC risk.
**Material and Methods:** DNA was extracted from peripheral blood cells of 1099 individuals: 305 patients histopathologically diagnosed with NSCLC and 794 healthy individuals without evidence of neoplastic disease. Genotyping of *TGFB1+869T>C* polymorphism was performed by Real-Time PCR allelic discrimination method. The odds ratio (OR) and its 95% confidence interval (CI) were calculated as a measure of the association between *TGFB1+869T>C* genotypes and NSCLC risk.
**Results:** The frequency of the TT genotype was lower in LC patients than in controls (29% and 37%, respectively). Conversely, we found an overrepresentation of C carriers in NSCLC group in comparison with normal controls (71% and 63%, respectively). Carriers of the C allele had an increased risk for NSCLC development (OR = 1.44, 95%CI = 1.07−1.94, $P = 0.012$). Stratification according to histological type, showed a statistical significant increased risk for epidermoid NSCLC development in the individuals with C carrier genotypes when compared to individuals with TT genotype (OR = 1.68, 95%CI = 1.05−2.72, $P = 0.023$).
**Conclusions:** Individual differences in cellular microenvironment may influence the susceptibility to cancer development and behaviour. Our results suggest that *TGFB1+869T>C* functional genetic polymorphism influence NSCLC susceptibility. This genetic profiling may help define higher risk groups for an individualized therapy.

**1095** POSTER

**Pharmacogenomic analysis of the triplet combination of gemcitabine, oxaliplatin and cetuximab as salvage therapy for metastatic colorectal cancer (mCRC) patients**

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**Background:** We have previouslyreported that the combination of biweekly gemcitabine-based therapy wasactive in pretreated mCRC pts (De la Cruz et al, ASCO GI 2008, abstr377). We aimed to investigate whether germ line polymorphisms may bepredictors of clinical outcome in mCRC pts treated with thiscombination.
**Material and Methods:** We evaluated SNPs ofgenes involved in gemcitabine metabolism (CDA, dCDK, RRM1, DCTD, SLC28A1), DNA repair (XRCC1, XRCC 3, ERCC1, XPD) and two IgG Fragment CReceptor polymorphisms (FcgRIIa- H131R and FcgRIIIa-V158F) reported tobe predictive of cetuximab-based therapy, even in K-ras mutatedpts. Whole blood was collected and DNA extracted from peripherallymphocytes using a DNA isolation Kit (Qiagen, CA). Polymorphisms weredetected using the TaqMan genotyping assays (Applied Biosystems, CA). Clinical response was evaluated according to RECIST criteria. Univariate analysis (Fisher's exact test for response; log-rank testfor TTP and OS) was performed to examine associations betweenpolymorphisms and clinical outcome.
**Results:** Blood samplesof 35 out of 39 enrolled pts were tested for genomic analysis. Patient's characteristics are as follows; M/F: 26/13, median age: 59 years, median number of prior chemotherapy lines: 2 (1−4), Köhne riskgroups; low: 8 pts, intermediate: 18 pts, high: 13 pts. After a medianfollow-up of 20 months, median progression-free survival (PFS) is 6.7months (95% CI; 5.2−8.3) and median overall survival 15.4 m (95% CI;14.7−16.1). Overall response rate (ORR) was 53.8%. RRM1 R284R ($p = 0.06$),T741T ($p = 0.02$) and RRM1–524CT ($p = 0.04$) were linked to clinicalresponsiveness. All pts possessing 2 or 3 favourable RRM1 SNPsresponded. ORR was 53.3% for pts with no favourable SNPs versus 85% forthose pts with any favourable SNP ($p = 0.04$). ORR was also significantlyhigher in pts with any histidine allele in the FcgRIIa polymorphism (93% vs. 60%, $p = 0.034$).
Median PFS was adversely affected in ptsharbouring no favourable RRM1 SNPs (4.2m versus 6.7 months, $p = 0.019$) and in those pts with homozygous FcgRIIa-131R allele (4.4 vs. 7.5months, $p = 0.007$).
**Conclusions**: Polymorphic variants of RRM1 and FcgRIIa may play a key role in the efficacy of gemcitabine-based therapy for mCRC pts.

**1096** POSTER

**Molecular signatures of disseminated tumour cells in metastatic breast cancer patients**

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**Background:** The critical step in breast cancer progression is the spreading of tumor cells to distant organs. Numerous studies have demonstrated that the presence of Disseminated Tumor Cells (DTC) in the bone marrow from patients with breast cancer is an independent prognostic factor for systemic relapse and breast cancer related death. Immunocytochemical methods for detection of DTC make it possible to identify single DTC in a population of normal cells. However, we still have limited knowledge about the biological and molecular characteristics of the DTC themselves. Complete genomic profiles and expression patterns have to be considered in order to understand tumor aggressiveness, clinical outcome and/or the disseminated tumor cell status.
**Material and Methods:** Mononuclear cells from the bone marrow of metastatic breast cancer patients are transferred to slides, DTC are identified by immunocytochemical staining and isolated by micromanipulation. Samples are amplified by single cell whole genome amplification and the resulting amplified DNA is applied to high density whole genome Agilent CGH arrays.
**Results:** We tested and established the Single Cell array Comparative Genomic Hybridization (SCaCGH) technique in our laboratories in order to investigate the molecular signatures of DTC in metastatic breast cancer patients. DTC from the bone marrow of metastatic breast cancer with variable numbers of DTC per mononuclear cells were selected and analyzed. In a first pilot of about 10 patients we compare the genomic profiles of 1−3 DTC per patient in relation to each other and among different patients. Our preliminary results show concordance of the genetic profiles from different DTC within a patient and different copy number variations among the DTC from different patients.
**Conclusions:** Due to the implementation of a technique called the Single Cell array Comparative Genomic Hybridization (SCaCGH) we are finally able to characterize single cells using high density microarrays. This will provide us with information about the properties of DTC important for the understanding of the metastatic cascade, apart from the potential to better understand tumor heterogeneity in general.

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**1097** POSTER

**Genetic polymorphisms of thymidylate synthase and DNA repair genes are associated with the toxicities of S-1 and cisplatin combination chemotherapy in metastatic or relapsed biliary tract cancer**

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**Background:** Biliary tract cancers (BTC) are often diagnosed at advanced stage and are still fatal in the majority of patients. Although the standard chemotherapy for metastatic or relapsed BTC is not established yet, we recently reported phase II trial which showed the efficacy of S-1 and cisplatin combination chemotherapy (Kim et al. Ann Oncol, 2008). In addition, it was suggested that genetic polymorphisms, especially genes related to 5-fluorouracil and cisplatin activity, may be associated with various toxicities of chemotherapy.
**Objective:** The aim of this study was to explore the relationship between the toxicity of S-1/cisplatin combination chemotherapy and the germline polymorphisms of genes associated with these agents including thymidylate synthase (TS), xeroderma pigmentosum group D (XPD), the excision repair cross-complementation 1 (ERCC1), and X-ray repair cross-complementing group (XRCC).
**Methods:** Ninety-four patients were received S-1 80 mg/m$^2$ day 1−14 and cisplatin 60 mg/m$^2$ on day 1 every 3 weeks. Genomic DNA was extracted from the mononuclear cells obtained from the patients before receiving S-1 and cisplatin. Polymorphisms were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.
**Results:** Median follow up duration was 22 months (95% Confidential Interval (CI): 11.1−22.9 months). Objective response rate was 33.4%. Median progression-free and overall survival were 5 and 9 months, respectively (95% CI: 1.2−5.8 and 7.1−10.9 months, respectively). Diarrhea was significantly more frequent in patients possessing TS 3'-untranslated region (UTR) 6bp deletion homozygote (42.6% in −6bp/−6bp vs. 20% in +6/−6 or +6/+6, *P = 0.021*). Grade 3−4 anemia was more frequently observed in patients with TS 3'-UTR 6bp deletion homozygote (20.4% in −6bp/−6bp vs. 5% in +6/−6 or +6/+6, *P = 0.038*). The C/C genotype of XPD-Arg156Agr was significantly associated with grade 3−4 neutropenia (50% in the C/C genotype vs. 23.5% in the C/A or A/A genotype, *P = 0.013*). The C/T or T/T genotype of XRCC1-Arg194Trp, the G/G genotype of XRCC1-Arg399Gln, and the C/C genotype of ERCC1-C8092A were significantly correlated with grade 3−4 thrombocytopenia (*P = 0.045*, *P = 0.039*, and *P = 0.037*, respectively).
**Conclusion:** Our results suggest that germline genetic polymorphisms in TS and DNA repair genes are associated with increased risk of toxicities in BTC patients who received S-1 and cisplatin combination chemotherapy.

**1098** POSTER

**Influence of IL-4 −590C/T polymorphism in Non-Small Cell Lung Cancer (NSCLC) susceptibility**

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**Background:** Lung cancer is the leading cause of death by cancer in the world, originating about 17.5% of total deaths from cancer (1.18 million). Inflammation and related pathways play an important role in the pathogenesis of lung cancer. IL-4 is an anti-inflammatory cytokine, which reduces the production of proinflammatory cytokines by monocytes and with direct antiproliferative effects in some tumors. The polymorphism *−590 C/T* SNP is a C to T transition in the *−590* position of the promoter region of the *IL-4* gene, and the T variant is associated with increased expression of IL-4. The aim of this study was to evaluate the influence of this polymorphism in the susceptibility to non-small cell lung cancer development (NSCLC).
**Methods:** DNA was extracted from peripheral blood of 696 individuals (277 patients diagnosed with NSCLC and a control group of 419 individuals without cancer). The characterization *IL-4 −590C/T* genotypes was performed by PCR-RFLP (*BsmFI*).
**Results:** The *−590 C/T* polymorphism genotypes were classified as low (CC) and high expression (TT). The frequencies obtained for the CC and TT genotypes were 86.3% and 13.7%, respectively, in the control group and 92.3% and 7.7%, respectively, in the case group. The analysis of the TT and CC genotype frequencies in the two groups under study showed a statistically significant difference in its distribution, indicating a protection of 48% for the development of NSCLC in individuals with the TT genotype when compared with individuals with CC genotype (P = 0.036, OR = 0.522: 95% CI = 0.282−0.965). This result is more pronounced when considering only the NSCLC epidermoid histological type (P = 0.003, OR = 0.086: 95% CI = 0.012−0.636). Stratifying according to smoking status, the results also show that smokers with the TT genotype have a protection for the development of NSCLC (P = 0.007, OR = 0.100: 95% CI = 0.013−0.772), when compared with the non-smoking group with TT genotype.
**Conclusion:** The results of this study point to the involvement of CC and TT genetic variants of the *IL-4 −590 C/T* polymorphism in the development of NSCLC. Increased expression of IL-4 associated with the TT genotype may contribute to the promotion of immune surveillance during NSCLC development, which could explain the results obtained in this work. However, further functional studies regarding IL-4 expression according to *IL-4 −590 C/T* polymorphism genotypes should be conducted in order to validate this hypothesis.

**1099** POSTER

**Influence of CXCR4 localization on in vitro migration of non small cell lung cancer cell lines and on clinical outcome in NSCLC**

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**Background:** Metastatic spread is the primary source of morbidity and mortality in non-small cell lung cancer (NSCLC). CXCR4, a G protein coupled chemokine receptor, and its ligand, stromal cell derived factor-1 (SDF-1), play a critical role in organ specific tumor metastasis. In vitro, CXCR4 expression has been shown to correlate with migration, invasion and adhesion. In vivo, patients whose tumors exhibit high CXCR4 expression have a poorer clinical outcome, while nuclear localization of the receptor specifically confers a better prognosis. We investigated the effect of CXCR4 localization on NSCLC cell migration and set out to quantify the expression of CXCR4 on tumor specimens from NSCLC patients to correlate CXCR4 localization with clinical outcome.
**Methodology:** CXCR4 localization was determined by cell fractionation and western blot, immunocytochemistry (IHC) and flow cytometry. Migration was investigated using a 48-well Boyden chamber. Demographic details, clinical variables and outcome data were gathered on patients diagnosed at the TBCC in 2004–2005. Formalin-fixed paraffin embedded tumor specimens (resected tumors Stage I and II; biopsies stage IV) were obtained and tissue micro arrays (TMA's) generated. CXCR4 expression was analyzed within lung cancer cells using the HistoRx PM-2000 platform. Statistical analysis was by Kaplan-Meier method, multivariate analysis and spearman's rank correlation.
**Results:** Inhibition of the this axis reduced cell migration. The degree of inhibition correlated with membrane localization of CXCR4. 790 patients were diagnosed with NSCLC at the TBCC in 2004–2005; 390 stage IV disease with 170 tissue samples available, 80 suitable for TMA generation; 85 early stage resected disease, all with suitable tissue. Preliminary analysis showed that the stage-based overall survival parallels results seen in other studies. The overall survival of patients whose tumors were suitable for TMA generation did not differ from the general cohort. Automated IHC for CXCR4 was successfully completed in all TMAs.
**Conclusions:** In vitro interruption of the CXCR4/SDF-1 axis inhibits the migration of NSCLC cells and correlates to membrane localization of CXCR4. Although the proportion of patients whose tissue is suitable for TMA generation makes up only 25−30% of all stage IV patients, this does not seem to introduce outcome bias in our cohort. CXCR4 expression is expressed in a large proportion of NSCLC tumors. Final results on the relationship between CXCR4 expression and outcome will be presented.

**1100** POSTER

**Peripheral blood lymphocite populations in advanced gastric cancer patients have predictive and prognostic value**

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**Introduction and Objectives:** Quantify in gastric cancer patients peripheral blood lymphocite populations levels at diagnosis time and their value as a treatment response predictor and as a survival prognostic factor.
**Patients and Methods:** Were eligible for this study irresectable/locally advanced and metastatic gastric cancer patients. Relapsed gastric cancer patients after radiacal surgical treatment were also eligible. No surgical,