Acknowledgements: This work has received research funding by the Communities Sixth Framework Programme as part of the international DISMAL collaboration for research into disseminated epithelial malignancies.

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

J. Rhee[1], Y.J. Kim[1], S.A. Im[1], S.W. Han[1], K.W. Lee[1], I.S. Choi[1], D.Y. Oh[1], J.H. Kim[1], T.Y. Kim[1], Y.J. Bang[1]. [1]*Seoul National University College of Medicine, Internal medicine, Seoul, South Korea*

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

M. Gomes[1], A. Coelho[1], A. Araujo[2], R. Catarino[1], A. Nogal[1], A.L. Teixeira[1], R. Medeiros[1]. [1]*Oncology Portuguese Institute, Molecular Oncology, Oporto, Portugal;* [2]*Oncology Portuguese Institute, Oncology Department, Oporto, Portugal*

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

S. Otsuka[1], H. Muzik[1], E. Stolte[1], W. Boland[1], A. Klimowicz[1], M. Konno[1], D. Morris[1], T. Magliocco[1], G. Bebb[1]. [1]*Tom Baker Cancer Centre, Medicine, Calgary Alberta, Canada*

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

R. Pazo Cid[1], J. Lao Romera[1], M. Lanzuela[2], I. Gil[1], A. Godoy[3], N. Fernandez-Mosteirin[3], J. Madani[1], M.J. Lecumberri[1], M. Alvarez Alejandro[1], A. Antón[1]. [1]*Hospital Universitario Miguel Servet, Medical Oncology, Zaragoza, Spain;* [2]*Hospital Universitario Miguel Servet, Radiation Therapy, Zaragoza, Spain;* [3]*Hospital Universitario Miguel Servet, Hematology, Zaragoza, Spain*

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,

chemotherapy or radiotherapy treatment was allowed in the 6 month period before entering the study. Peripheral blood populations phenotype was analyzed by flow citometry (CD4+, CD8+, CD19+, CD56+/CD16+).
Results: Between February 2007 and April 2008 sixty advanced gastric cancer patients were tested; median age 65 years old; medium Karnofsky index 70%; 91% of the patients had normal CD19+ B lymphocite peripheral blood levels; 95% of patients had T-lymphopenia of any grade.
T CD4 lymphopenia: observed in 96% of patients (medium level 482.75 CD4/ml): in localized gastric cancer patients medium CD4 levels (574.38/ml) were higher than in metastatic gastric cancer patients (390.17/ml) ($p = 0.049$). A statistically significative difference ($p < 0.003$) in CD4 levels was detected when comparing Karnofsky Index $\geqslant$80% patients (medium 668.90 CD4/ml) and KI $\leqslant$ 70% patients (medium 371.48 CD4/ml). If less than 520 CD4/ml median survival was 6 months and response rate to treatment was 25%; 11 month median survival and 40% response rate to treatment when patients had CD4 levels greater than 520/ml ($p < 0.002$).
T CD8 lymphopenia: detected just in 55% of patients (medium 980.24 CD8/ml); different peripheral blood CD8 levels if localized gastric cancer (media 592.46/ml) or metastatic gastric cancer (387.78/ml) ($p = 0.049$). No diferences were detected in CD8 levels when analyzing KI, response rate to treatment or survival.
Conclusions: Worse response rate to treatment and poorer survival outcome is observed in gastric cancer patients that at diagnosis time have peripheral blood CD4 levels lower than 520 CD4/ml.

1101 POSTER

Prostate cancer and apoptosis: An insight of FAS-670A/G polymorphism role in tumor development

L. Lima[1], A.M. Gomes[1], R. Ribeiro[1], A. Fraga[2], F. Pina[3], F. Lobo[4], F. Calais-da-Silva[5], R. Medeiros[6]. *[1]Portuguese Institute of Oncology, Molecular Oncology Unit, Porto, Portugal; [2]D. Pedro V Millitary Hospital, Urology Department, Porto, Portugal; [3]S. João Hospital, Urology Department, Porto, Portugal; [4]Portuguese Institute of Oncology, Urology Department, Porto, Portugal; [5]Central Region Hospital, Urology Department, Lisbon, Portugal; [6]Portuguese Institute of Oncology, Molecular Oncology Group, Porto, Portugal*

Background: Apoptosis is an essential process in the elimination of malignant cells. One of the characteristics of malignant cells and of tumor development is tumoral cell evasion to apoptotic stimuli and alterations of the apoptotic pathways components.
FAS-670A/G polymorphism in the promoter region of *FAS* gene has been identified as possible role in prostate cancer development. In this study we present an insight of these findings, with a large sample and a wider analysis.
Methods: We performed Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) methodology, for *FAS* gene locus −670 genotyping. It was evaluated DNA samples from 1056 men with prostatic disease: 874 prostate cancer patients and 182 Benign Prostatic Hyperplasia (BPH) patients.
Results: We found that the presence of GG genotype of *FAS-670* A/G represents a significant protection for advanced disease – T3/T4/N+/M+ (odds ratio (OR) = 0.52; confidence interval (CI): 0.32−0.86), and metastatic disease – N+/M+ (OR = 0.16; CI: 0.05−0.44). Moreover, we found that individuals carrying *FAS-670* GG genotype had a protection for the development of biochemical recurrence (OR = 0.35; CI: 0.13−0.90) and hormone resistance (OR = 0.22; CI: 0.06−0.76).
A linear trend analysis was performed and the results revealed an augmented protection with the *FAS-670* G allele number increase for advanced disease ($p = 0.013$) and biochemical recurrence ($p = 0.011$).
We also found that patients with *FAS-670* GG genotype have lower PSA levels when compared with *FAS-670* AA individuals ($p = 0.015$).
Conclusions: It was proposed that *FAS-670* G allele may reduce sFas levels preventing the apoptotic inhibition caused by the soluble form. Therefore, our results indicate that *FAS-670A/G* may have an important role in prostate cancer development, possibly due to the influence in apoptosis.

1102 POSTER

Chemotherapy increases HLA-ABC expression on tumour cells and promotes allogeneic cytotoxic responses

W.M. Liu[1], P. Smith[1], D. Fowler[1], A.G. Dalgleish[1]. *[1]St George's University of London, Oncology Division of CMM, London, United Kingdom*

Background: Evasion of immune surveillance is a hallmark of cancer. One level of immune surveillance is provided by the human leucocyte antigen class I system (HLA1), which is down-regulated in some tumours rendering them undetectable by immune cells. As part of our ongoing studies to investigate the impact of conventional chemotherapy on immune function, we explored the effect of chemotherapy on HLA1 expression on tumour cells. Our hypothesis was that restoration of HLA1 expression on tumour cells may re-engage immune-cell function and promote tumour cell death.
Materials and Methods: The tumour cell lines A549 (lung), Caki2 (renal) HCT116 (colon), MCF7 (breast) and PC3 (prostate) were cultured for 3-days with equi-active concentrations of the chemotherapy drugs cyclophosphamide (10 μM), gemcitabine (1 μM) or oxaliplatin (5 μM). HLA1 levels were assessed before and after treatment. We also investigated the effect that changes to HLA1 expression may have had on the ability of cytotoxic T-cells (CTLs) to induce death, by subjecting HLA-1 modified tumour cells to a modified mixed lymphocyte reaction. To this end, we co-cultured tumour cells with allogeneic CTLs, and assessed cytotoxicity after 24 h by using the LDH and MTT assays.
Results: HLA1 expressions (mean fluorescence intensity (MFI) relative to isotype controls) ranged from 8.5±0.29 in A549 to 27±5.1 in Caki2, and separated into cells with low expression (A549 and MCF7) and those with high (Caki2, HCT116 and PC3). Culturing cells with cyclophosphamide or oxaliplatin had little impact on HLA-1. However, culturing with gemcitabine resulted in significant increases in expressions in HCT116, A549 and MCF7 cells (MFI cf. untreated controls: 132±30 vs. 33±7.8;.23±2.3 vs. 10±0.67; 45±11 vs. 18±3.7, respectively; $p < 0.01$). Parenthetically, basal expression was low in two of the cell lines. Crucially, in cell lines with increased HLA-1 expression, there were clear reductions in cell number and concomitant increases in cell death (increase in cytotoxicity: 53%, 120% and 94%, in HCT116, A549 and MCF7, respectively). Cytotoxicity appeared to be HLA-1-mediated as inhibiting HLA-1 with a blocking antibody reduced the extent of the cell death.
Conclusions: These results provide evidence that a facet of immune surveillance can be restored by chemotherapy, which results in increased CTL activity. This supports our overall notion of improving cancer therapy through the use of chemotherapy as immune modulators.
This work was supported by the Cancer Vaccine Institute charity (http://www.cancervaccine.org.uk/)

1103 POSTER

Supernatant from tumour cells treated with chemotherapy stimulate professional antigen presenting cells in vitro

D. Fowler[1], W.M. Liu[1], P. Smith[1], A.G. Dalgleish[1]. *[1]St George's University of London, Cellular and Molecular Medicine, London, United Kingdom*

Background: The maturation of dendritic cells (DCs) is an important element of the adaptive immune response. DCs process and present foreign elements to T-cells and thereby initiate antigen-specific T-cell responses. Their activity is controlled by cytokines. As part of our ongoing studies to investigate the impact of chemotherapy on immune function, we tested the hypothesis that chemotherapy-stressed tumour cells secrete cytokines that promote the antigen presenting behaviour of DCs.
Materials and Methods: DCs were generated from plastic-adhered monocytes using a 7-day culture with 100 nG/mL GM-CSF and 50 nG/mL IL-4 q.o.d. The tumour cell lines A549 (lung), HCT116 (colon) and MCF7 (breast) were cultured for 3-days with equi-active concentrations of cyclophosphamide (C: 10 μM), gemcitabine (G: 1 μM) or oxaliplatin (O: 5 μM). Supernatants were removed and DCs cultured in them for 24 h before phenotyping for CD80, CD83 and CD86 as a way to assess DC maturation and stimulation.

	CD80		CD83		CD86	
	%+ve	MFI	%+ve	MFI	%+ve	MFI
medium	77	19	1.0	11	19	12
C	74	22	0.61	23	13	16
G	76	25	0.39	31	12	17
O	70	21	0.52	34	13	16
tumour	64	10	1.4	23	36	7.4
tumour+C	77	11	2.7	13	43	9.6
tumour+G	68	53	21	34	69	37
tumour+O	74	43	8.6	29	58	29

Results: Our plastic adherence method of DC-generation resulted in high yields (~80% − based on FSC and SSC patterns), and the purities of the DCs (CD11c+/HLA-DR+) were >95%. Monocyte contamination was low with an average CD11c+/CD14+ signal of 1.5%. Culturing DCs with chemotherapy alone resulted in changes to CD80, CD83 and CD86 as defined by both %positive cells (%+cells) and mean fluorescence intensities (MFI). These changes were not significantly different to those seen after culture with basal medium. Although there were significant increases in these differentiation markers on culturing DCs with supernatant derived from tumours, there were further increases in expressions when the