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

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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

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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.
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1103 POSTER

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

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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