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

supernatant were from tumour cells treated with chemotherapy. This was most clearly seen when using A549 cells (Table).
Conclusions: These results provide evidence that tumours exposed to some chemotherapy release cytokines that can mature DCs and ultimately enhance T-cell responses. This supports our overall notion of improving cancer therapy through the use of chemotherapy as immune modulators.
This work is funded by the Cancer Vaccine Institute Charity (www.cancervaccine.org.uk).

1104 POSTER

Immunological response to influenza vaccine in cancer patients undergoing treatment with sunitinib or sorafenib

S.F. Mulder[1], J.F.M. Jacobs[2], M.A.M. Olde Nordkamp[2], I.K. Kremer[1], P.F.A. Mulders[3], C.J.A. Punt[1], I.J.M. de Vries[2], C.M.L. van Herpen[1]. *[1]Radboud University Nijmegen Medical Centre, Medical Oncology, Nijmegen, The Netherlands; [2]Radboud University Nijmegen Medical Centre, Tumor Immunology, Nijmegen, The Netherlands; [3]Radboud University Nijmegen Medical Centre, Urology, Nijmegen, The Netherlands*

Background: It is recommended to annually vaccinate against influenza persons at risk for complications from influenza (i.e. chronic diseases accompanied with immunosuppression and/or age $\geqslant$60 years). Sunitinib is commonly used in the treatment of advanced renal cell cancer (RCC) and gastro-intestinal stroma cell tumours (GIST), and sorafenib in RCC and hepatocellular cancer. Sunitinib and sorafenib have immunomodulatory effects in both human and murine models, with largely unknown clinical consequences. In this study we investigated the efficacy of influenza vaccination in terms of immune responses in cancer patients on sunitinib or sorafenib treatment.
Materials and Methods: In the autumn of 2008, four different groups were vaccinated with subunit trivalent influenza vaccine. Group 1 (RCC) and group 2 (GIST) were pts on treatment with sunitinib or sorafenib for $\geqslant$4 weeks. Group 3 were advanced RCC pts without treatment for $\geqslant$1 year. Group 4 were healthy volunteers age $\geqslant$60 yrs. Eligibility criteria included an indication for influenza vaccination, no corticosteroid use in the last 2 weeks, and no immunotherapy or targeted therapy in the last year, except for imatinib in pts with GIST. Peripheral blood mononuclear cells (PBMCs) and serum was collected prior to (day 1) and on day 8 and day 22 after influenza vaccination. Humoral immune responses were measured with antibody titers against all three influenza strains in the vaccine. PBMCs were re-stimulated with the influenza strains in-vitro, to measure vaccine specific, cell-mediated immune responses. As a read-out we measured the PBMC proliferation, activation and cytokine production.
Results: 40 subjects were enrolled (group 1: n = 19, median age 61 yrs, group 2: n = 3, 53 yrs, group 3: n = 7, 60 yrs, group 4: n = 11, 66 yrs). No serious side effects of the vaccinations were observed. Preliminary results show a vaccine-specific humoral immune-response in all groups. Updated results on both humoral and cellular responses will be presented.
Conclusions: During treatment with sunitinib or sorafenib cancer patients can mount a humoral response against the influenza vaccine. Our data suggest that these vaccinations can be recommended in this population.

1105 POSTER

Tumour growth suppression of mouse colon cancer cell line by Boron neutron capture therapy & Dendritic cell derived immunotherapy

H. Yanagie[1], K. Kakimi[2], A. Hosoi[2], A. Ogata[3], Y. Sakurai[3], Y. Morishita[4], A. Shinohara[5], H. Sugiyama[3], M. Eriguchi[3], H. Takahashi[1]. *[1]The University of Tokyo, Department of Nuclear Engineering & Management, Tokyo, Japan; [2]The University of Tokyo Hospital, Department of Immunotherapeutics (Medinet), Tokyo, Japan; [3]The University of Tokyo Hospital, Cooperative Unit of Medicine & Engineering, Tokyo, Japan; [4]The University of Tokyo, Department of Human & Molecular Pathology, Tokyo, Japan; [5]Jyuntendo University, Department of Hygenes, Tokyo, Japan*

Background: The cytotoxic effect of Boron neutron capture therapy (BNCT) is due to a nuclear reaction between ^{10}B and thermal neutrons. The short range in tissue of the resultant lithium ions and a particles (5–9 mm) restricts radiation damage to those cells in which boron atoms are located on neutron irradiation. Dendritic Cells (DCs) are potent antigen-presenting cells, so it is now being focused on the role of DCs in eliciting antitumour immunity and in potential therapeutic applications. Recently, it has been reported that combined immuno and radiation therapy results in effective tumour growth suppression. In this study, we prepared the cationic liposome (COATSOME-EL) as the effective ^{10}B carrier into the cancer cells, and we evaluated the synergic anti-cancer effects of immune-responces with dendritic cells intratumoural injection after BNCT.
Materials and Methods: Sodium salt of ^{10}B compound ($Na_2{}^{10}B_{12}H_{11}$ SH; BSH) (10 mg/ml) solution was added to the COATSOME EL-C-01. On day 10, when the tumours of Colon26 reached an average diameter of 10 mm, BNCT on mice IT injected with ^{10}B entrapped liposome (150 μl) was performed with thermal neutrons (2×10^{12} n/cm^2) at JRR4 reactor of Japan Atomic Energy Research Institute, and, at that time, syngeneic DCs (1×10^7 cells/mouse) were injected IT with 3 times, or 7 times. After BNCT+DCs injections, the effect of treatment was calculated on the basis of tumour volume and morphological findings of the tumours at 4-day intervals.
Results: Significant tumour growth suppression was achieved on the group treated BNCT + IT-saline, and the combination of BNCT + IT-DC compared to non-treated group, and 30% tumour growth suppression was achieved on 7 times IT-DC group. Splenocytes retrieved on day 40 after tumour inoculation from mice subjected to the BNCT+ 7 times DCs showed significantly more tumour-specific IFN-γ-secreting cells compared with splenocytes from control groups. In tumor challenged mice, that received spleen cells from BNCT+DCs treated mice, Fifty % reduction in tumour growth was observed.
Conclusions: Our data indicate that DC administration combined with BNCT induces tumour antigen-specific cellular-mediated immune responses in tumour bearing mice. DCs combined with BNCT of a solitary tumour confers protection against tumour rechallenge. We hope to apply this direct DC immunotherapy for enhancing the BNCT effects in clinical trials.

1106 POSTER

Cognitive study of reactivity to IPH1101 of peripheral gamma delta T lymphocytes from solid tumour patients

F. Rolland[1], D.R. Propotnich[2], B. Escudier[2], J. Bennouna[3], E. Bompas[3], H. Sicard[4], A. Collette[5], C. Paiva[6], S. Lafaye de Micheaux[7]. *[1]Centre René Gauducheau, Oncology, Saint Herblain, France; [2]Institut Medical Montsouris, Oncology, Paris, France; [3]Centre René Gauducheau, Oncology, Saint Herblain, France; [4]Innate Pharma, gd T cells platform, Marseille, France; [5]Texcell, Laboratories, Evry, France; [6]Innate Pharma, Clinical Operations, Marseille, France; [7]Innate Pharma, Clinical Program Development, Marseille, France*

Background: IPH 1101 is a chemically-synthesized structural analogue of natural phosphoantigens recognized by a population of non-conventional lymphocytes bearing potent anticancer activity known as gamma-delta (γδ) T lymphocytes. IPH 1101 combined with low doses of IL-2 induces a highly selective proliferation of γδ T cells.
IPH1101 associated with low doses of IL-2 has been tested in phase I clinical trials in various solid tumours and Non-Hodgkin Lymphoma patients, showing a good safety profile and significant target lymphocyte population amplification. IPH 1101 is currently being tested in Phase II clinical trials in haematological indications (alone or in combination).
The objective of this prospective *ex vivo* observational study is to explore potential indications in which IPH1101 may be beneficial. Because cancer patients may have impaired immune function, it appears important to assess the proliferative capacity of the patients' γδ T cells in response to IPH 1101 + IL-2.
We have set up a quantitative standardized *in vitro* "IPH1101 sensitivity test" that requires only a small sample of patients' peripheral blood mononuclear cells (PBMC).
We report here results from selected solid tumour indications: renal cancer (RCC), colorectal cancer (CRC), prostate cancer (PC), bladder cancer (BC) and lung cancer (LC).
Material and Methods: Patients (pts) with RCC, CRC, PC, BC, and LC were enrolled at 2 French sites. A small sample of blood (20 mL) was sufficient to prepare PBMCs and culture them in the presence of IPH1101 and IL-2. Results on the extent of *in vitro* amplification of cells by IPH1101 were available within 8 days and were expressed as (i) % of γδ T cells in the culture and (ii) total amplification rate of γδ T cells.
Results: 59 pts with RCC (of which 20 were under TKI treatment), 30 pts with CRC, 41 pts with PC, 19 pts with BC and 6 pts with LC were evaluable in this study. Patients presented various type of tumour, stage of disease (most of them were metastatic) and various prior treatment types. All pts but one were found sensitive to IPH1101 stimulation ex vivo and more than 90% of them presented a high level of response to this test.
Conclusion: Taking into account their preserved γδ Tcells expansion functions, patients suffering from renal, colorectal, prostate, bladder, and lung cancers might be candidate for future studies with γδ T cells immunomodulators.