regarding “picking up the pieces” of their life before cancer. (eg, Ganz et al. 2004, Cimprich et al, 2005).

Various different studies have examined ways of making the progression from the end of adjuvant treatment easier for patients. Psycho-educational groups have been proposed as an effective method of easing the transition.

Methods: A literature review identified the topics that patients describe as difficult to cope with, effective formats of intervention and the predictors of distress at the end of treatment. A focus group was conducted with a sample of patients who had finished active trreatment in the previous year. The focus group was structured by the findings of the literature review, but participants were also able to comment freely on their own experiences.

The results of this were compiled with the evidence base and a six session group programme was developed. Each week would consist of a psycho-educational slot covering a different topic, with guest speakers, followed by a therapeutic session. The topics highlighted by the literature and the focus group included diet and exercise, relaxation, managing emotions, family relationships, returning to work and preparation for ongoing symptoms.

The group was evaluated using the Hospital Anxiety and Depression Scale and the Mental Adjustment to Cancer Scale, administered pre and post group. This would be followed up at six months post group to assess whether improvements were maintained.

Participants were also asked to feedback their own feelings about the effect of the group.

Results: The overall usefulness of the group was rated on a Likert Scale of 0 = not useful, to 10 = extremely useful. The average rating from the group was 8.4 (n=23) indicating members had subjectively found it very beneficial.

The group was shown to be beneficial in all areas of assessment pre and post, including anxiety, depression and mental adjustment (n=23). (Up to date data will be presented)

Conclusion: Preliminary results are encouraging and suggest that patients find a combination of psychoeducation and psychotherapeutic support to be beneficial at this point in their cancer journey. Results will become more robust as further data is collected but it is hypothesised that the improvements in mood and mental adjustment will be sustained.

626 Poster

Modulation of PKC delta and epsilon distribution by plant phenols in mouse epidermis

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Protein kinase C (PKC) is thought to be a major intracellular receptor for the mouse skin tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). PKC is a serine-threonine-specific kinase and represents a family of at least 11 isozymes, which can be divided into three main groups: the Ca+2-dependent or conventional PKCs (alpha, beta and gamma), the Ca+2-independent or novel PKCs (delta, epsilon and ni) and atypical PKCs (zeta). The diversity of PKC isoforms and their central role in many signaling pathways makes them important targets for potential chemopreventive agents. Our previous studies showed that three structurally diverse phenolic acids: protocatechuic acid, chlorogenic acid and tannic acid and trihydroxystilbene – resveratrol, altered the TPA-stimulated distribution and activity of PKC alpha, beta, gamma and zeta in mouse epidermis. Their effect on other PKC isozymes: delta and epsilon, might be of interest since transgenic mice with over-expressed PKC delta showed resistance to tumor promotion by TPA, while over-expression of PKC epsilon caused a reduction in the pappiloma burden, although enhanced carcinoma formation. Better understanding of different epidermal expression of PKC isoform patterns and substrate proteins is needed to explain their opposing effects on skin carcinogenesis and its modulation by plant phenols.

Thus, the aim of current study was the evaluation of the effect of plant phenols on TPA-stimulated PKC delta and epsilon distribution. Phenolic acids and resveratrol were applied topically at the dose of 16 micromoles 15 minutes before a single application of 3.4 nmoles of TPA in acetone. Control mice were treated with acetone only. Forty eight hours after TPA treatment animals were sacrificed and the cytosolic and particulate fractions were isolated. The distribution of PKC isozymes was determined by Western blot analysis.

TPA treatment resulted in the translocation of both estimated PKC isozymes from cytosolic to particulate fractions. All tested phenolic compounds affected the TPA-induced PKC isozymes translocation. The observed effects, however, were depended on the phenol structure and to a certain extent were isozyme specific. Protocatechuic acid and chlorogenic acid significantly inhibited the TPA stimulated translocation of PKC delta. For PKC epsilon the similar effect was observed after treatment with chlorogenic acid, tannic acid and resveratrol.

The results of the present study may point out the significant role of PKC isozymes in the promotion of mouse skin tumorogenesis by TPA and suggest that antipromotional activity of plant phenols may result from the modulation of PKC isozymes distribution, including PKC delta and epsilon.

627 Poster

Immunohistochemical study of intratumoral microvessels in resected non-small cell lung carcinomas, N-status, pTNM-stage and survival period of the patients

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Goal: Study of intratumoral microvessels in resected non-small cell lung carcinomas (NSCLC), N-status, pTNM-stage and survival period.

Material and method: Resected material from 54 patients radically operated for NSCLC is observed. 21 cases concern N0-status, 33-N1,2 – status. 48 cases concern I, II and IIIA, and 6-IIIB and IV pTNM stage. The number of intratumoral microvessels (NITMV) is determined through application of CD31. There is an account of high (NITMV=>75), and a low (NITMV<75) degree of vascularisation. Intratumoral vessel invasion is determined. Statistical methods: t-test, chi-square, survival according to Kaplan-Meier, logistic regression analyses.

Results: The average survival period in low vascularisation is 1731 days, and in high vascularisation – 1158 days (a 573 days difference, p=1067). NITMV has statistically significant influence on the N-status: chi-square-p=0.041, logistic regression analyses – p=0.045. A significant dependency between the average NITMV and pTNM stage (p=0.029) has been proven. In vessel invasion (in 27.4% of the cases) the survival period is shorter with 478 days. In 28 cases (54.9%) intratumoral vessels immediately bordering tumor cells are observed, while in 5 NSCLC there are intratumoral vessels, in part of whose walls endothelial cells are not found.

Conclusion: NITMV has a statistically significant influence on the N-status. The survival period is longer in NSCLC with low vascularisation.

POSTER SESSION

Tumour immunology

628 Poster

Regulatory T cells are recruited and activated within primary breast tumors with an adverse clinical outcome

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Background: Breast Cancer (breast adenocarcinoma) is the most common cause of cancer in women in developed countries and the second leading cause of cancer death in women. Clearance of primary breast tumors by immune mechanisms is rare despite the fact that some of them contains T cells infiltrates. Regulatory T cells (Treg) are increased in peripheral blood of patients with breast cancer and present in tumor environment. In this work we assessed the role of Treg in breast tumor progression.

Materials and methods: Immunohistochemical analysis of Foxp3 expression by TMA and ex-vivo analysis of Tumor-infiltrating Treg (Ti-Treg) were performed on patients suffering from primary breast carcinoma.

Results: Immunohistochemical analysis of Foxp3 expression in primary human breast tumors showed that the presence of Ti-Treg within the tumor bed had no influence on tumor progression in opposition to Ti-Treg within lymphoid infiltrates that was predictive of relapse and death, in particular in ER+ patients. Moreover, our ex-vivo analyses demonstrated that these tumors are highly infiltrated by $CD4^+CD25^{high}CD127^{low}Foxp3^+$ Ti-Treg that suppress the functions of conventional T cells (Tconv). Ti-Treg are selectively recruited through CCR4 or CCR7 as suggested by their down-regulation at cell-surface and the presence of their ligands in tumor environment. Furthermore, Ki67 and Hoechst 33342 stainings demonstrated their local expansion. Importantly, in contrast to Ti-Tconv and circulating Treg, Ti-Treg expressed high levels of GITR, ICOS, HLA-DR,

demonstrating that they are selectively activated locally, and, suggesting specific recognition of tumor-associated antigens.

Conclusion: Altogether our results demonstrate that Treg are selectively recruited within breast tumors and are activated within lymphoid infiltrates containing mature dendritic cells (DC), resulting in immune escape through Tconv inhibition and ultimately tumor progression. As we previously described that plasmacytoid DC infiltration in human breast tumors was also correlated with an adverse clinical outcome (Treilleux et al, 2004), studies are in progress to investigate the interactions between PDC and Treg within breast tumors.

629 Poster

The WT1 antigen as a novel target for human leukemia-specific CD4+ T regulatory T cells

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Background: Recent studies demonstrated that regulatory T cells (Tregs) play an important role in regulating immune responses in cancer patients. The Wilms tumor antigen (WT1) is overexpressed in several cancers and it has been considered as a potential target for cancer immunotherapy. However, the generation of an effective anti-WT1-specific T cells has recently been shown to be largely affected by the presence of Tregs. We asked whether an anti-WT1 Tregs population exist in leukemia patients which may contribute to the impairment of anti-WT1 responses.

Materials & Methods: We used a pool of 110 WT1-derived peptides and a micro-scale WT1-peptide-set containing each peptide to identify an anti-WT1 Tregs epitope.

Results: We identified a Tregs population that specifically recognized a WT1-derived peptide (WT1-84) in an HLA-DRB1*0402/TCR-V beta 8-restricted fashion. These Tregs recognized HLA-DRB1*04-matched fresh leukemic cells expressing the WT1 antigen, exerted a Th2-cytokine profile, and had a CD4+CD25+Foxp3+GITR+CD127- Treg-phenotype. They significantly inhibited the proliferative activity of allogeneic MLR independently of cell-contact or cytokine production. Moreover, priming of allo-reactive T cells in the presence of Tregs strongly inhibited the expansion of NK; NK-T and CD8+ T cells; had an inhibitory effect on NK/NK-T cytotoxic activity but not on CD8+ T cells. The generated Tregs specifically produced Granzyme-B but not perforin and selectively induced apoptosis in WT1-84 pulsed-autologous APCs. Granzyme B produced by Tregs can induce apoptosis in target cells. Importantly, preliminary data indicated that anti-WT1-84 Tregs may exist in HLA-DR4-matched leukemia patients.

Conclusions: These findings will have important implications for the clinical manipulation of Tregs.

630 Poster

The prognostic value of intraepithelial and stromal innate immune system cells in non-small cell lung carcinoma

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Background: The major value of prognostic markers in potentially curable non-small cell lung carcinoma (NSCLC) should be to guide therapy after surgical resection. In this regard, the patient immune status at the time of resection may be important and also measurable. The immune system has paradoxical roles during cancer development. However, the prognostic significance of tumor infiltrating macrophages, natural killer (NK) cells and dendritic cells is controversial and not thoroughly studied, especially in the tumor stroma. The aim of this study is to elucidate the prognostic significance of these cells in the epithelial and stromal compartments of NSCLC.

Materials and Methods: Tissue microarrays from 335 resected NSCLC, stage I-IIIA were constructed from duplicate cores of viable and representative neoplastic epithelial and stromal areas. Immunohistochemistry was used to evaluate cells in epithelial and stromal areas with respect to CD68 (macrophage marker), CD56 (NK cell marker) and CD1a (dendritic cell marker).

Results: In univariate analysis, increasing numbers of stromal CD1a+ cells (P = 0.011) and CD56+ cells (P = 0.014) correlated significantly with an improved disease-specific survival (DSS). No such relation was noted for CD68+ cells or for epithelial CD1a+ and CD56+ cells. The prognostic significance of stromal CD56+ cells was an independent prognostic factor for DSS, P = 0.031 (HR 2.337, C.I. 1.081-5.049).

Conclusions: High density of stromal CD56+ cells is an independent factor associated with a better prognosis in resected NSCLC, suggesting that these cells might mediate a strong antitumor immune response in the tumor stroma.

631 Poster

CD44 promotes repopulation of thymus and T cell maturation in allogeneic bone marrow transplantation

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Background: Allogeneic bone marrow cell reconstitution (BMC) can provide an ultimate therapy in patients with hematological malignancies and solid tumors for which T progenitor cell homing into the thymus and maturation is of particular importance. In search for improving this protocol, we explored the impact of CD44 standard (pre T cell marker) and variant isoforms CD44v6 and CD44v7 on progenitor T cell homing and maturation.

Materials and methods: Progenitor cell homing into the bone marrow and the thymus was studied through short term (CFSE labelling) and long term reconstitution experiments. Prolifereation and apoptosis assays were performed with (H3)thymidine incorporation and Annexin V staining. In order to study the effect of CD44 on each subpopulation of thymocytes cells were sorted into double negative (CD4-CD8-), double positive (CD4+CD8+) and single positive (CD4+/CD8+) thymocytes with magnetic beads.

Results: CD44 has a major impact on progenitor cell homing into the bone marrow and the thymus. Antibody blocking studies and the transfer of CD44v7-deficient (CD44v7-/-) BMC provided evidence that bone marrow homing is also influenced by stromal cell CD44v7. Homing into the thymus was CD44v6 and CD44v7 independent. However, CD44v6 supported thymocyte expansion and apoptosis resistance. CD44v6 induced apoptosis resistance most strongly in double negative cells that was accompanied by Akt activation and Bcl-2 up regulation. In addition, CD44v6 induced proliferation of double negative thymocytes that proceeded via activation of the MAPK pathway. Distinct to early thymocytes, in double positive and single positive thymocytes CD44v6 only supported signal transduction via the TCR/CD3 complex.

Conclusions: Thus, CD44 plays a major role in hematopoietic stem cell homing and survival and is also required for thymus homing. CD44v6 in particular supports survival and expansion of early progenitor T cells. Accordingly, the transfer of CD44v6 transfected T progenitor cells can be expected to accelerate the reestablishment of a competent and host tolerant immune system.

632 Poster

Lentiviral TCR gene transfer for adoptive immunotherapy of cancer

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The immune system is often unable to mount effective T cell responses against tumours because tumour-associated antigens are poorly immunogenic. The introduction of alpha and beta chain genes of a specific TCR into T cells, has been shown a very promising therapy. The current clinical translation of this approach is based on gene transfer with retroviral vectors. However, TCR gene transfer using retroviral vectors can be achieved only after in vitro polyclonal stimulation of the target T cells, which may result in exhaustion and terminal differentiation. Lentiviral vectors are an attractive alternative to allow TCR gene transfer in the absence of polyclonal activation that may improve subsequent adoptive T cell therapy by maintaining naive phenotype and improved homing characteristics of gene modified T cells.

Lentiviral vector constructs have been generated containing both chains of an HLA-A*0201-restricted TCR specific for Wilms` tumour antigen 1 (WT1), myeloid leukaemias associated antigen. We analysed the effect of common gamma chain receptor cytokines IL2, IL7, IL15,IL21 on the transduction efficiency, proliferative potential, phenotype and functional activity of the WT1 TCR-transduced T cells. Primary T cells were successfully transduced after treatment with low-dose common gamma chain cytokines, either individually or in combination. All cytokines tested promoted the maintenance of a naïve phenotype as shown by expression of CD28 and CD62L. IL21 has shown to be important for homeostasis of naïve T phenotype of the transduced T cells. We hypothesize that more undifferentiated TCR-transduced T cells may demonstrate improved functional avidity in vivo than terminal differentiated T cell obtained by