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.

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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

polyclonal activation and transduction with retroviral vectors. In vivo experiments are in progress to determine whether in vivo survival of transduced T cells can be influenced by the in vitro transduction method.

633 Poster

Expression pattern of IL-1 beta in malignant and benign prostate tissues coincides with its inhibitory effects on carcinoma cells

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Although overproduction of IL-1beta is often associated with aggressive growth of many tumors, data on expression of this cytokine in neoplastic and benign prostate tissue are limited. In our study performed on archived samples of prostate carcinoma (N=28, Gleason scores 6-9) and age-matched control benign hyperplastic tissue (BPH, N=30), an overall IL-1beta expression was lower in cancer relative to BPH (p=0.037). In BPH, IL-1beta was detected only in stroma, where its levels positively correlated with intensity of lymphocyte infiltrate (p=0.043). In low-grade cancer, IL-1beta was apparent in stroma and malignant glands, while high-grade tumors were negative. In cancer foci, stromal IL-1beta levels positively correlated with its glandular levels (p=0.017); both glandular and stromal levels negatively correlated with tumor grade (p=0.003) implying that IL-1beta exerts inhibitory effects on cancer cells. Indeed, IL-1beta-treated androgen-responding LNCaP carcinoma cells exhibited time- and dose-dependent growth delay accompanied by redistribution of cells to G1 phase of the cell cycle. Although cells displayed an increase of cytosolic cytochrome C and mitochondrial Bak, no cytotoxicity was detected. However, pretreatment of LNCaP cells by IL-1beta markedly increased their susceptibility to etoposide-induced apoptosis with both caspase 2 and caspase 8 involved. IL-1beta also stimulated its own secretion in LNCaP cells. The experiments employing co-cultures composed of cytokine-pretreated and -not-treated cells or utilizing IL-1 receptor antagonist and media conditioned by IL-1-pretreated cells demonstrated that this amplification loop created by IL-1beta was essential for rendering these cells more susceptible to apoptosis. Amplification loop was also detected in androgen-insensitive PC3 and DU-145 carcinoma cells; however, proliferation and apoptotic resistance of these cells were not affected. In the contrast, non-neoplastic PWR-1E prostate cells failed to secrete IL-1beta even when pretreated by the cytokine. These cells were highly susceptible to etoposide-induced apoptosis; no further increase in apoptosis was observed in IL-1beta-pretreated cultures relative to controls treated by etoposide only. Identified tissue expression patterns considered together with growth-retarding and pro-apoptotic activities exerted by IL-1beta on androgen-responding carcinoma cells indicate that IL-1beta may attenuate the initial steps of prostate cancer.

634 Poster

miRNAs expression profiles during ErbB2 driven mammary carcinogenesis

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Since the expression of miRNAs is modulated during cancer development, miRNA profiles provide new classifications of human cancer. The epigenetic silencing of genes plays important roles in tumor evasion from the immune control: miRNAs are involved in regulation of the immune system and in the generation of T regulatory cells. In view of the roles played by miRNAs in cancer progression, we performed a miRNA microarray analysis aimed at identifying the modulation of miRNA expression profiles during the progression of authoctonous mammary carcinomas arising in mice transgenic for the activated transforming rat ErbB2 oncogene (BALB-neuT mice). BALB-neuT mice constitute a suitable cancer-prone model, since inexorably the females develop an invasive and metastatic mammary cancer in all of their ten mammary glands with a step wide pattern and a systemic metastatic spread similar to that observed in human mammary cancer. We analyzed three prototypic situations: i) normal hyperplasia (2 weeks pregnant BALB-c mice), ii) atypical hyperplasia (10 weeks old BALB-neuT mice) and iii) neoplastic lesion (19 weeks old BALB-neuT mice). Total RNA was extracted from the all mammary glands from 4 animals for each group of mice and miRNAs changes within these three prototypic situations were evaluated using LNA microarrays (EXIQON A/S, Denmark). Linear model analysis was performed in order to find out subsets of probes differentially expressed between the mammary glands of 10 and 19 weeks old BALB-neuT mice with respect to pregnant BALB/c mice. The principal component analysis (PCA) done on 169 miRNA, indicated a significant difference between miRNAs expressed in the mammary tumors of BALB-neuT females and those expressed in the mammary glands of pregnant BALB/c females. This result suggest that the expression of miRNAs during physiological mammary hyperplasia and neoplastic hyperplasia is different. By contrast, when the analysis was performed to evaluate the differences between mammary atypical hyperplasia and neoplastic lesions, PCA showed only subtle differences suggesting that miRNA disregulation in the mammary gland of BALB-neuT females is an early event which is then stably maintained during the tumor progression.

635 Poster

Silencing IL-10 gene with intra-mammary siRNA enables DNA vaccination to inhibit established ErbB2 carcinomas

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In this study we intended to locally perturb tumor microenvironment in order to circumvent peripheral tolerance and induce an effective and persistent immune response.

We have shown that a DNA vaccine coding for the extracellular and transmembrane (EC-TM plasmid) domain of rat-ErbB2 (neu) halts the early stages of carcinogenesis in neu transgenic BALB/c mice (BALB-neuT). These mice develop mammary carcinomas that closely resemble human carcinomas in both tumor progression and immune tolerance to ErbB2, and in the expansion of suppressor cells. Marked expansion of CD11b+GR1+ immature myeloid cells and CD4+ CD25+ FOXP3+ GITR+ Treg cells during tumor progression in BALB-neuT mice makes immunotherapy against advanced lesions ineffective.

IL-10 is a key figure among the various tumor-derived and tumor induced factors that contributes to damp down the immune response. It is produced by both tumor cells and by cells with immunosuppressive activity such as T reg cells, tumor associated macrophages, tolerogenic DC and immature myeloid cells.

By using intra-nipple delivery it is possible to get a direct access to breast lesions and target immune suppressor cells that localize in the mammary tumor microenvironment. We have employed this technique to silence IL-10 gene in mammary glands of 18-week-old BALB-neuT mice with established lobular carcinomas. Intra-nipple injection of 50ug of IL-10 siRNA plasmid was followed 2 days later by intramuscular electroporation with 50ug of EC-TM plasmid. While EC-TM vaccination alone was only able to slightly delay the appearance of the first palpable tumor, prior silencing of IL-10 gene in the tumor microenvironment enabled the vaccine to elicit a protective anti-tumor immune response founded on a high titer of anti-neu antibodies and a significant citotoxicity activity against Erbb2 peptides. All the mice that received both treatments were tumor free at 33 week of age, when all untreated mice, or mice treated with control IL10 siRNA or EC-TM vaccine alone were already dead.

In conclusion, this experiment planned to selectively perturb tumor microenvironment through intra-nipple injection of IL-10 siRNA may prove a new promising way of circumventing tolerance and eliciting protective immunity against established lesions.

636 Poster

Probing the molecular mechanism of GD2 ganglioside mimicry with 12-amino acid constrained peptides

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Aberrant glycosylation is an universal feature of cancer cells. Over-expression of GD2 ganglioside (GD2) on neuroblastoma (NB) cells opens the possibilities to use the tumor associated carbohydrate antigen in immunotherapy. Moreover, the fact that about 60% of children with neuroblastoma have high risk tumors, which can rarely be cured by conventional therapy, stresses the need for new treatment protocols to control minimal residual disease.

Our goal is to design GD2-targeting active immunotherapy of NB by replacing the weakly immunogenic GD2 with its peptide mimotopes. We have identified 12-amino acid (aa) peptides that can mimic GD2 by screening the LX-8 phage-displayed peptide library with the GD2 specific mouse monoclonal antibody (mAb) 14G2a. In the current study, we have explored the observed GD2 mimicry phenomenon by vaccination studies in mice. Additionally, we have designed peptides with aa substitutions. In the next step, we have analyzed their 14G2a mAb binding in competition tests against GD2-expressing human NB cells, and microplate-bound GD2 using flow cytometry and ELISA, respectively.

We show that vaccines containing our peptides conjugated to KLH can induce GD2-targeting antibodies in A/J mice. Additionally, we have further