characterized the molecular mechanism of GD2 mimicry observed with our isolated peptides by application of alanine scanning experiments. This has allowed us to determine the involvement of consecutive aa residues of the peptides in the 14G2a mAb binding. Using competition assays we have identified the aa residues that are critical for the binding. Furthermore, in an attempt to optimize the GD2 mimotopes we have designed and characterized a peptide sub-library containing aa substitutions at the pivotal positions for the 14G2a mAb binding. Finally, we have screened the peptides for their ability to bind to mAb specific for other gangliosides.

The accumulated data allowed us to gain insight into the molecular mechanism of GD2 ganglioside mimicry by the mimotopes. This can lead to increase of therapeutic potential of our GD2 mimotopes. More research is planned to optimize the GD2-specific immune responses induced with the mimotopes, by testing their anti-tumor activity on a NB model based on the A/J mouse strain and syngenic NXS2 cells.

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An MVA based vaccine targeting the oncofoetal antigen 5T4 in patients undergoing surgical resection of colorectal cancer liver metastases

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Purpose: This study investigated the use of a therapeutic vaccine, TroVax? in patients undergoing surgical resection of colorectal cancer liver metastases. Systemic immunity generated by vaccination before and after resection of metastases was measured in addition to assessing safety and toxicity and analyzing the function and phenotype of tumour associated lymphocytes,.

Experimental Design: Twenty patients were scheduled to receive 2 TroVax vaccinations at 2 week intervals pre-operatively and 2 post-operatively; if immune responses were detected 2 further vaccinations were offered. Blood samples were taken at trial entry and 2 weeks after each vaccination; tumor biopsies were taken at surgery. 5T4-specific cellular responses were assessed by lymphocyte proliferation and ELISPOT, while antibody responses were measured by ELISA. Immunohistochemistry was used to characterize antigens expressed on the tumour and to analyze the phenotype of infiltrating lymphocytes.

Results: Twenty patients were recruited, one of whom was found to have hepatocellular carcinoma. Of the 19 colorectal cancer (CRC) patients, seventeen showed 5T4 expression in the tumour or surrounding stroma and 18 mounted a 5T4-specific cellular and/or humoral response. In patients where surgery was at least potentially curative (n=15), those with above median 5T4-specific proliferative responses or T cell infiltration into the resected tumour showed significantly longer survival compared to those with below median responses. A similar, but non-significant, trend was also associated with the 5T4 antibody response.

Conclusion: These data suggest that the magnitude of 5T4 (but not MVA) specific antibody and proliferative responses and the density of CD3 cells in colorectal cancer liver metastases are associated with clinical benefit. Such encouraging observations warrant more extensive studies to identify the precise underlying mechanisms.

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Tumor antigen NY-CO-58/KIF2C is strongly overexpressed in a variety of human cancers and evokes spontaneous T cell responses

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INTRODUCTION: A recent study indicated that NY-CO-58/KIF2C might be overexpressed in colorectal cancer. However, NY-CO-58/KIF2C expression has not been examined in detail in this tumor type, little is known about the expression of NY-CO-58/KIF2C in other cancers, and it is unclear whether this tumor antigen is able to induce spontaneous T cell responses in cancer patients.

METHODS: We examined the expression of NY-CO-58/KIF2C in colon cancer cell lines, a broad series of healthy human tissues, and malignant as well as autologous healthy tissues from patients with colorectal cancer (N=22). In addition, normal and tumor-infiltrated samples from patients with pancreatic (N=17), gastric (N=10), head-and-neck (N=30), and breast cancer (N=44) were examined for NY-CO-58/KIF2C expression using conventional RT-PCR, real-time PCR, Western blot, immunofluorescence, and immunohistochemistry. Finally, we analyzed peripheral T cells of 43 patients with colorectal cancer and 35 healthy controls for responses against nine 30mer peptides of NY-CO-58/KIF2C following one cycle of antigen-specific stimulation.

RESULTS: Colon cancer cell lines strongly expressed NY-CO-58/KIF2C on the RNA and protein levels. Among 20 normal tissues, human testis expressed the highest levels of NY-CO-58/KIFC, thymic tissue showed an intermediate level, and the remaining healthy tissues only evidenced trace levels of NY-CO-58/KIFC. Examining samples of patients with colorectal cancer using real-time PCR, we found that NY-CO-58/KIF2C was strongly overexpressed in the malignant compared to autologous healthy colon tissue. Immunohistochemisty localized NY-CO-58/KIF2C expression to malignant epithelial tissue. Analyzing malignant and autologous healthy tissues from patients with pancreatic, gastric, breast, and head-and-neck cancer, we found that NY-CO-58/KIF2C was significantly overexpressed in all these tumor types. CD8+ T cell-mediated responses were only detected in less than 10% of patients or healthy controls and were generally weak. In contrast, we found CD4+ T cell responses against one or more NY-CO-58/KIF2C peptides in close to 50% (20/43) of patients with colorectal cancer. Surprisingly, we observed equally frequent NY-CO-58/KIF2C-specific CD4+ T cell responses in the healthy blood donors with the majority (21/35) of subjects evidencing a response against at least one NY-CO-58/KIF2C peptide. Importantly, NY-CO-58/KIF2C-specific CD4+ T cells were of high avidity, recognized the naturally processed antigen, and secreted Th1-type cytokines.

CONCLUSION: Based on its overexpression in a number of human cancers and its high immunogenicity we suggest that NY-CO-58/KIF2C represents an attractive target for active tumor immunotherapies.

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Tumor antigen-encoding mRNA for the analysis of spontaneousand vaccine-induced immune responses in cancer patients

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Background: For the development of effective cancer vaccines there is a requirement for the assessment of vaccine induced immunity. Current immunomonitoring strategies do not allow for the optimal investigation of the full breadth of T cell responses, and is hampered by the limited number of known epitopes for most tumour antigens.

Methods: In this study transfection of antigen-presenting cells (APC) with modified mRNA constructs encoding for tumour antigens was optimized. mRNA encoding for full length NY-ESO-1 and CT-7/MAGEC1 has been applied to monitor T cell responses in cancer patients with naturally occurring immune responses to their tumour or following vaccination.

Results:CD8 T cells obtained from lung cancer patients with humoral immune responses directed towards NY-ESO-1 could be successfully amplified in vitro following only one stimulation round with mRNA-transfected APC. Specific killing of a panel of HLA-matched allogeneic NY-ESO-1 expressing tumour cell lines by the monoclonal CD8 T cells indicates an oligoclonal response including a novel HLA-B49 restricted epitope. Detection of NY-ESO-1 specific CD4 T cells in patients could be enhanced using a modified mRNA construct that targets the MHC class II pathway. The establishment of functional CD4 T cell clones specific for NY-ESO-1 has enabled the definition of the restriction element HLA-DQB10301 and HLA-DPB10402. Oligoclonal CD8 and CD4 T cell responses were detected in patients following an NY-ESO-1 vaccination. Using a modified CT-7 encoding mRNA, CT-7 specific CD4 T cells were detected in melanoma patients.

Conclusion: This methodology allows for a more precise monitoring of responses to tumour antigens in a setting that addresses the breadth and magnitude of antigen-specific T cell responses, and that is not limited to a particular combination of known epitopes and HLA-restrictions.

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Epigallocatechin-3-gallate inhibits monocyte adhesion and migration to sites of inflammation

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Monocytes/macrophages play an important role on initiation, development, and outcome of the immune response. Epigallocatechin-3-gallate (EGCG), a major component of green tea, has been reported to have antiallergic and anti-inflammatory activities. Our group demonstrated previously that EGCG

inhibits monocyte chemotactic protein-1 (MCP-1) in the human mast cell line HMC-1, decreasing its abiliy of monocyte recruitment, but the effects of EGCG directly on monocytes has not yet been explored. This work shows that EGCG decreases monocyte migration ability in response to MCP-1 and inhibits MCP-1 secretion and CCR2 expression, the specific receptor for MCP-1, using the human monocyte cell line THP-1. Moreover, EGCG has been described to inhibit the expression of some integrins. Our work demonstrates that EGCG decreases the levels of integrin beta1 activated, one of the primary integrins that can assembly monocytes to extracellular matrix under normal conditions, and THP-1 adhesion to fibronectin. We conclude that this study support the effects of EGCG as an anti-inflammatory compound.

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641 Poster

High excretion of etheno adducts in liver fluke-infected patients: protection by praziquantel against DNA damage

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Background: Chronic infection by liver fluke (Opisthorchis viverrini, OV), is a strong risk factor for developing cholangiocarcinoma (CCA). To clarify the involvement of oxidative stress and lipid peroxidation (LPO)–derived DNA damage, the excretion of LPO-derived etheno DNA adducts was measured in urine samples collected from healthy volunteers and OV-infected Thai subjects.

Materials and Methods: The study was performed in healthy volunteers (n=20, 9 males and 11 females) and OV-infected subjects (n=50, 26 males and 24 females). Urinary 1,N^6-etheno-2'-deoxyadenosine (εdA)- and 3,N^4-etheno-2'-deoxycytidine (εdC)-levels were quantified by immunoprecipitation-HPLC-fluorescence detection and ^{32}P-postlabeling thin-layer chromatography. Urinary malondialdehyde (MDA) was measured by the thiobarbituric acid-based method. Urinary nitrate/nitrite was measured by a simple Griess-based method. Plasma alkaline phosphatase (ALP) activity, a marker of hepatobiliary tract damage, was analyzed by a standard automated spectrophotometer using a commercial kit.

Results: Excreted etheno adduct levels were related to indicators of inflammatory conditions, MDA-, nitrate/nitrite-levels in urine and plasma ALP activity. Mean εdA- and εdC-levels were 3-4 times higher in urine of OV-infected patients; MDA, nitrate/nitrite and ALP were also increased up to 2-fold. MDA and ALP were positively related to εdA excretion. Two months after a single dose of the anti-parasitic drug praziquantel, εdA and εdC concentrations in urine of OV-infected subjects were decreased; MDA, nitrate/nitrite and ALP were concomitantly lowered.

Conclusions: We conclude: chronic OV-infection through oxidative/nitrative stress leads to massive urinary excretion of the etheno-bridged deoxy-ribonucleosides, reflecting high LPO-derived DNA damage in vivo. These promutagenic DNA etheno-adducts in bile duct epithelial cells may increase the risk of OV-infected patients to later develop CCA. Urinary εdA and εdC levels should be explored (i) as non-invasive risk markers for developing opisthorchiasis-related CCA and (ii) as promising biomarkers to assess the efficacy of preventive and therapeutic interventions.

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Tumor associated antigens identify a high risk benign disease group

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Benign breast diseases appear in more than half of all women after 20. Although a history of benign breast disease (BBD) indicates some increase in breast cancer, only a fraction develops malignant disease. The relationship between benign breast diseases and cancer development remains a subject of controversy. The aim of the present report is to detect associated tumor antigens in 80 tissue samples belonging to BBD. Samples were classified in three risk groups depending on proliferation: without or minimal proliferation: no risk benign disease (NRBD); increased proliferation: low risk (LRBD) and atypical epithelial hyperplasia: high risk (HRBD). An immunohistochemical study was performed employing the following antibodies: anti-MUC1 protein core (C595, HMFG2 and SM3 monoclonal antibodies, MAbs), anti-MUC1-cytoplasmic tail (MUC1-CT) polyclonal antibody (Ab) (CT33), anti-MUC4 Ab, anti-MUC2 MAb (PMH1) and anti-carbohydrate associated antigens MAbs: sialyl Lewis x (KM93), Lewis x (KM380), Lewis y (Lewis y) and Tn antigen. Statistical analysis: Frequencies Analysis and Multiple correlation including principal components analysis (PCA) were performed. Results: In NRBD: MUC1 was detected in 62,9% with C595, 27,4% with HMFG2 and 17,2% with SM3. In LRBD, the percentages were: 53,3%, 50% and 31,3%, respectively while in HRBD: 50%, 50% and 16,7%, respectively. MUC1-CT percentages were: 80%, 93,8% and 50%, respectively. Lewis x was the carbohydrate antigen more frequently found in the three groups while sialyl Lewis x were less found (0% in HRBD); on the contrary, Lewis y was more expressed in HRBD than in the other risk groups. MUC2 was also mainly detected in HRBD while MUC4 in LRBD. A statistical significant ($p<0.05$) correlation between anti-MUC1 protein core MAbs and anti MUC1-CT Ab was found ($r=0,7373$). PCA explained 86% of data variability (risk groups and tumor antigenic expression); PCA first two components grouped all HRBD patients while LRBD and NRBD remained spread. Conclusions: 1- HRBD express a determine pattern of tumor associated antigens employed in this study and 2- In BBD, anti-MUC1 CT was the more useful MAb to detect MUC1 showing a high correlation with anti-MUC1 protein core MAbs.

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The immunoproteome of pancreatic cancer

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The purpose of our study is to identify novel tumour antigens by characterising a panel of proteins related to pancreatic cancer. These antigens may be used as tools for early diagnosis, prognosis and some may be good targets for cancer vaccine development.

In developed countries, pancreatic cancer is the fifth leading cause of cancer death. This cancer form is difficult to diagnose even at more advanced stages of the disease with prognosis very poor due to limited treatments offered. Thus, tools for early diagnosis and prognosis as well as new therapeutic agents are essential. Patients diagnosed with pancreatic cancer develop antibody responses against pancreatic tumour related proteins, also called tumour antigens, during the course of the disease. We utilised this and conducted an autologous SEREX analysis involving phage display and automated high-throughput screening of a cDNA tumour library made from a pancreatic cancer patient.

We identified cDNAs encoding 11 different identities. The dominating cDNAs encoded insulin, a hypothetical protein and NADH dehydrogenase (ubiquinone) flavoprotein 1. Other identities were the chromosome 19 open reading frame 60, keratin 19, calcineurin binding protein 1, coiled-coil domain containing 85B, heterogeneous nuclear ribonucleoprotein, TIMP metallopeptidase inhibitor 1, interferon alpha-inducible protein 27 and ADP-ribosylation-like factor 6 interacting protein 4. Furthermore, we found that of 37 pancreatic cancer patients examined 33% had autoantibodies against insulin whereas the percentage was 16% for healthy donors.

The antigens identified from the pancreatic cancer patient reflect a well known relationship between pancreatic cancer and diabetes since some of the antigens are related to the cancer development such as interferon alpha-inducible protein 27 and TIMP metallopeptidase inhibitor 1 and some e.g. insulin are related to diabetes. Due to the broad expression of the majority of the identified antigens they may not be targets for immunotherapy but further evaluation of the antigens will determine their diagnostic value.

644 Poster

Development of monoclonal antibodies for the identification of novel invasion associated targets in human cancer

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Monoclonal antibodies (MAb's) have emerged as an ever increasingly important tool in cancer therapy. They have yielded promising results when used alone or in combination with current therapies. The development of MAb's also allows for the discovery of novel cancer associated antigens. In this study, monoclonal antibodies were generated by immunising Balb/c mice with the invasive melanoma cell line MDA-MB-435S/F and an invasive variant of the MiaPaCa pancreatic cell line. Following fusion, all resultant hybridomas were screened against their respective target immunogens using newly developed screening systems based on live cell immunofluorescence and a 96 well based invasion assay (Boyden chamber) system. MAb 7B7 has been shown to inhibit invasion (up to 50% of control level), and motility (up to 70% of control level). MAb 9E1 inhibits invasion (up to 70% of control level), but not motility. A dose response inhibitory effect on invasion has also been observed with MAb 7B7.