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