("late vaccination"). In conclusion, our data suggest that the combination of Treg temporary interference with ErbB2 specific DNA tumor vaccine reshuffles the T cell repertoire, and lead to both preventive and therapeutic anti-tumor immunity.

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Methods for the isolation and identification of MHC-presented peptides from leukaemic cells

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Aberrant kinase activity is implicated in the majority of malignancies. As specific immune responses may be generated to phosphopeptides presented by MHC molecules, the isolation of these peptides from the surface of cancer cells may form a basis for immunotherapy. In chronic myeloid leukaemia (CML) the majority of cases (95%) are caused by the fusion of the bcr and abl genes which results in the production of a deregulated tyrosine kinase, hypothetically resulting in aberrant MHC phosphopeptide expression.

Here we describe the development of a mild acid elution technique to selectively release peptides from MHC class I or II complexes, with minimal contamination by intracellular material or serum proteins. Unlike previous approaches, this method is compatible with immobilised metal ion affinity chromatography (IMAC), a powerful tool for fractionation of peptides and the subsequent simplification of mass spectrophotometric profiles. Fractionation of cell surface eluates from the CML cell line K562-A3 by a range of methods including IMAC and characterization by tandem mass spectrometry lead to the identification of numerous peptides and phosphopeptides, many of which bear strong links to malignancy. These include peptides from: ephrin-A4 precursor, elongation factor 1-alpha 1, MYEOV, and Myc binding protein 2; and phosphorylated peptides from nuclear receptor coactivator 2 and membrane-associated protein HEM-1.

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Analyses of novel tumour antigens as targets for cancer immunotherapy

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The efficiency of current immunotherapy approaches is still far from that expected. In order to identify immunogenic proteins of melanoma and prostate cancer that could be used as target genes for cancer therapy we screened phage-displayed cDNA libraries derived from melanoma prostate cancer, and testis with sera from 76 cancer patients. This resulted in the identification of >1000 different clones, however, only about 10% of them represented cDNAs fused in-frame to the T7 phage coat protein thus ensuring the exposition of natural products of genes on the phage surface. The rest likely represent mimotopes, albeit, there might be true tumour antigens among them that resulted from frame-shifting mutations or defective regulation of alternative splicing or translation.

As none of the identified clones contained a mutation we further looked for novel cancer-germ cell or overexpressed antigens. The criteria for selecting an antigen for expression analyses were: it (1) contains an uncharacterised splice variant, (2) represents a novel gene, (3) shows a cancer associated EST profile or (4) plays an important role in oncogenesis. The expression was tested in a panel of 15 normal tissues and in paired cancerous and adjacent normal tissues of 46 melanoma, breast, prostate and gastric cancer patients using qPCR. We have so far tested the expression of 18 antigens. We saw a testis specific expression for 2 of 3 genes with cancer associated EST profile, but no overexpression in cancerous tissues comparing to the normal counterparts was observed. 2 of 3 novel genes showed a predominant expression in testis and one so far tested also showed a cancer associated overexpression (clone 284). None of the 5 tested functionally relevant genes showed an elevated expression in cancer. 4 out of 7 novel splice variants of known genes were testis specific, and one of these tested so far (clone 29) showed also a cancer-associated overexpression.

The alternative splicing of immuno-privileged tissues like testis is very extensive, hence it is possible that due to splicing defects often observed in cancerous tissues such testis-associated isoforms could be formed and recognised by the immune system, leading us to hypothesise that analogously to cancer-germ cell expressed antigens a category of cancer-germ cell spliced antigens might exist. Clones 29 and 284 will further be subjected to T cell activation assays to test their potential to be used in cancer immunotherapy applications.

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CD8+ cytotoxic T lymphocytes generated against a WT1 peptide analog enhance the lytic activity of leukemic cells

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Background: The WT1 antigen performs an oncogenic function in various types of cancer. It is overexpressed in human leukemias and therefore it has been considered as an attractive target for immunotherapy. Most WT1-specific CTLs described displayed a low avidity and exerted minimal lytic activity against cancer cells.

Materials & Methods: We used an approach to improve the immunogenicity of CTL epitopes consisting of substituting the first-amino-acid, of 2 known HLA-A0201-restricted WT1-derived peptides (Db126 and WH187), with tyrosine (Y).

Results: This modification resulted in the enhancement of the binding ability of the 126Y analog and CTL generated against this peptide exerted a significantly lytic activity against the 126Y peptide-loaded target cells and importantly cross-reacted with the 126N native peptide. Another interesting finding is the significant high lytic activity recorded for the 126Y CTL against freshly isolated HLA-A0201-matched leukemic cells expressing the WT1 antigen. This data confirms that T cells generated against the 126Y analog peptide cross-react also with the naturally processed 126N native peptide. Moreover, it seems that stimulation with the peptide analog induced CTLs with a high TCR avidity. Finally, the high lytic activity provoked by the 126Y CTL may be also attributed to the significant high number of anti-126 T cell frequencies in this T cell line as demonstrated by IFN-gamma production in the ELISPOT assay.

Conclusions: This study provide evidence that peptide modification results in a better immune response against cancer and further support the use of this strategy as a potential approach for the development of a leukemia-vaccine.

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Study of Lewis y expression and anti Lewis y immune response through Lewis y-circulating immune complexes detection in breast cancer patients

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The aims of this study were: 1-to detect Lewis y (Ley) antigen in breast tumor samples; 2- to determine the role of MUC1 as a carrier of Ley and 3- to investigate the induction of humoral immune response through the detection of circulating immune complexes (CIC) carrying Ley in breast sera. Materials and methods: 137 breast tissue and serum samples: 72 malignant tumours, 30 benign diseases and 35 normals. The expression of Ley was determined by standard immunohistochemistry (IHC); percentage of stained cells, intensity and pattern of the reaction were analyzed. An immunoprecipitation was performed in order to determine if MUC1 may behave as a possible carrier for Ley. HMFG1, an anti MUC1 monoclonal antibody (MAb) was used to precipitate MUC1 from breast cancer sera. Immunoprecipitates (IP) obtained were run in SDS-PAGE and Western blot (WB) assays. Sheets were incubated with C14 (anti Ley) and HMFG1. An ELISA was developed to study the presence of Ley/CIC. Briefly, C14 was adsorbed in multiwell microplates and incubated overnight at 4ºC. After washing, 1% bovine serum albumin/PBS was added for 3 hours at 37ºC. Serum samples were incubated overnight at 4ºC and 1:2000 anti human IgM or 1:3000 IgG reacted with the complexes and revealed with ABTS and 30% H2O2 in sodium citrate buffer, pH 5.0; OD was measured at 405 nm. Results: By IHC with C14, positive results were found in 34% malignant tumors; 33% benign diseases and 35% normal samples; no statistical difference was found. The pattern of expression differed between malignant and non malignant samples: cancer specimens showed more frequently a cytoplasmic and membrane non apical reaction while non malignant samples showed an apical membrane reaction. By WB, IP displayed a band at >200KDa with both C14 and HMFG1 MAbs. By ELISA, mean OD for IgM/LeyCIC in breast cancer sera, benign and normal samples were: 0.538, 0.949 and 0.942, respectively. By ANOVA, significant statistical differences between breast cancer and normal and benign samples were found. Mean OD for IgG/LeyCIC were: 0.414, 0.438 and 0.492,