

Conclusions: This preliminary study seems to indicate that the presence of -765C COX2 allele could be an important marker to yield the identification of higher risk individuals for bladder cancer development that might benefit from chemopreventive strategies with COX inhibitors.

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POSTER

Novel epitopes presented by the HLA-A*0201 are recognized by the cytotoxic T lymphocytes of breast cancer survivors

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Cellular immune mechanisms detect and destroy cancerous and infected cells via the human leukocyte antigen (HLA) class I molecules that present peptides of intracellular origin on the surface of nucleated cells. The identification of novel, tumor-specific epitopes is a critical step in the development of immunotherapeutics for breast cancer. In order to directly identify peptide epitopes unique to cancerous cells, secreted human class I HLA molecules (sHLA) were constructed by deletion of the transmembrane and cytoplasmic domain of HLA A*0201. The resulting sHLA-A*0201 was transferred and expressed in breast cancer cell lines MCF-7, MDA-MB-231, and BT-20 as well as the immortal, non-tumorigenic cell line MCF10A. Stable transfectants were seeded into bioreactors for production of >25 mg of sHLA-A*0201. Peptides eluted from affinity purified sHLA were analyzed by mass spectroscopy. Comparative analysis of HLA-A*0201 peptides revealed 5 previously uncharacterized epitopes specifically presented by the tumorigenic cell lines. These peptides are derived from intracellular proteins with either well defined or putative roles in breast cancer development and progression: Cyclin Dependent Kinase 2 (Cdk2), Ornithine Decarboxylase (ODC), Kinetochore Associated 2 (KNTC2 or HEC1), Macrophage Migration Inhibitory Factor (MIF), and Exosome Component 6 (EXOSC6). From a small HLA-A*0201 positive cohort including 8 breast cancer survivors and 9 controls, cellular recognition of the ODC, MIF, KNTC2, EXOSC6, and Cdk2 peptides by circulating CD8+ cells from 3 cancer survivors was demonstrated by tetramer staining, IFN- γ ELISPOT, intracellular cytokine staining, and CD107a cytotoxicity assays. The identification and characterization of peptides unique to the class I of breast cancer cells provide putative targets for the development of immune diagnostic tools and therapeutics.

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POSTER

Screening Plasma Thioredoxin-1 (Trx-1) to potentially guide clinical development of the Trx-1 Inhibitor PX-12

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Background: PX-12, an irreversible thioredoxin (Trx-1) inhibitor, is currently being evaluated in a number of clinical trials. Its molecular target Trx-1 is over-expressed in many human cancers and is associated with aggressive tumor growth, poor prognosis and contributes to resistance to chemotherapy. Tumors which over-express Trx-1 also secrete the protein, and plasma Trx-1 levels of cancer patients have been found to be elevated over that of healthy individuals. After surgical removal of tumor burden, these plasma levels drop to the normal range. In previous and ongoing clinical trials, in some patients treated with PX-12, circulating levels of Trx-1 were lowered and a Trx-1 decrease of 25% or greater was associated with increased survival in the Phase I study. This current study evaluated the plasma of 135 patients from 7 different indications to determine if there may be potential to select patients based upon thioredoxin levels.

Methods: Frozen plasma samples were obtained from consented patients through the NCI's Cooperative Human Tissue Network and included 17–20 from each of prostate, renal, melanoma, endometrial, sarcoma, pancreas and colon cancers. Plasma Trx-1 protein was measured by a sandwich ELISA developed by Oncothyreon. Other angiogenic proteins regulated by Trx-1 were also evaluated by standard commercial ELISA. Trx-1 in ng/mL was determined for each samples and the data is reported by indication with comparisons to other proteins such as VEGF and FGF-2.

Results: A total of 135 patient samples from 7 different indications were evaluated for Trx-1, VEGF, FGF-2 and other angiogenic proteins. It was found that 37 of the 135 had elevated circulating levels of Trx-1 (>3-fold of the previously determined normal donor median, 5.4 ng/mL). Of the indications where samples were obtained, sarcoma, melanoma, pancreatic and endometrial had the highest percentage of patient plasmas with elevated Trx-1 values (50%, 45%, 35%, and 32% respectively). The plasma

Trx-1 levels will be presented along with trends with other Trx-1 controlled plasma protein and patient status information where available.

Conclusions: It has been found that patients with elevated plasma Trx-1 treated with PX-12 were more likely to have lowering of Trx-1 levels and possibly a better clinical outcome than patients with plasma Trx-1 in the normal range. The results from the current study show that approximately 30% of patients across a number of indications have elevated plasma Trx-1. The data suggests clinical studies of a Trx-1 inhibitor such as PX-12 may benefit from patient selection.

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POSTER

Promoter hypermethylation of the NORE1A occurs in endometrial cancer

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Background: Inactivation by promoter hypermethylation of the tumor suppressor gene RASSF1A and NORE1A have been reported in a variety of human cancers. Activating point mutations of the K-ras oncogene are a common feature of many tumor types.

Materials and Methods: We have analysed the K-ras mutational status and promoter hypermethylation of RASSF1A, NORE1A and hMLH1, and MSI of 78 endometrial cancers.

Results: Promoter hypermethylation of RASSF1A, NORE1A and hMLH1 was detected in 29.5% (23 of 78), 20.5% (16 of 78) and 26.9% (21 of 78) of endometrial cancers respectively. The absence of hMLH1 methylation and the absence of microsatellite instability (MSI) were almost exclusively confined to those without RASSF1A methylation in endometrial cancers respectively in this study. We demonstrate that RASSF1A promoter methylation was more frequently present in wild-type K-ras, and that NORE1A methylation was exclusively confined to those without hMLH1 methylation in the endometrial cancer.

Conclusions: Our results suggest that RASSF1A, NORE1A and hMLH1 are inactivated in a subset of endometrial cancers. The mutual exclusivity of the epigenetic and genetic alterations in these genes suggest that they play a critical and cooperative role in the carcinogenesis of endometrial cancer.

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POSTER

TM9SF2, a novel cell surface potential target in breast cancer

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Cell surface proteins of tumor tissues are believed to have different expression from their counterparts of normal tissues. Thus, cell surface proteins can be attractive therapeutic targets important to optimize treatment strategies. To detect differentially expressed cell surface markers in breast carcinoma, we analyzed gene expression profiles of eight human breast cancer cell lines and normal breast tissue with oligonucleotide microarray. The array chips were fabricated in our laboratory so that the design of probes was implemented primarily to include the DNA sequence coding the transmembrane region or the GPI-anchor attachment site; excluding any alternative splicing possibility. Besides, probes of control RNAs were allowed to hybridize to confirm the reliability of the hybridization reactions. We followed a two-level analysis for the study of this complex gene expression data. This analysis summarizes the data by the SOM component plane, and then clusters the SOM to investigate the feature gene expression patterns. The SOM reduced the nine dimensions of the data to cluster the genes on a two-dimensional sphere and thereby allows to easy display the data and reveal the gene expression patterns. As a result, we detected genes whose expression was significantly consistent in all the cancer cell lines used and higher than that of normal breast tissue. The genes were transmembrane 9 superfamily protein member 2 (TM9SF2), vitelliform macular dystrophy 2-like protein 3 (VMD2L3), adenylate cyclase type III (ADCY3), membrane-spanning 4-domains subfamily A member 2 (MS4A2), and short transient receptor potential channel 5 (TRPC5). Also, CD24 and ErbB3 were significantly upregulated; evidence that supports the integrity of both arrays and samples. We selected TM9SF2 which is related to cell-to-cell signaling and functions as a channel or small molecule transporter; along with CD24 and ErbB3 as monitors, to validate their expression levels by real time quantitative PCR. The gene expression levels of TM9SF2 were significantly higher in all carcinoma than in normal breast tissue. TM9SF2 protein expression was evaluated by immunofluorescence and it showed concordance with the PCR results. Treatment by siRNA has enhanced TM9SF2 knockdown and less viability of MDA-MB-231 cells when compared to those treated with scrambled control siRNA. Here on, more siRNA treatments are to be done on the other cell lines.