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Mucin gene (MUC1) transfected dendritic cells as tumor vaccine in patients with breast or pancreatic cancer

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The glycoprotein mucin, encoded by the gene MUC1, is a tumor antigen expressed on breast and pancreatic carcinoma cells. Due to an underglycosylation of tumor cells mucin core protein epitopes are exposed to the immune system and can be recognized by human cytotoxic T cells (CTL). However, T cell recognition of mucin core protein epitopes in patients is very poor and does not lead to an effective tumor eradication. This could be due to the lack of costimulatory molecules such as B7.1 and B7.2 on tumor cells. We therefore transfected MUC1 into human dendritic cells (DC's) expressing such costimulatory molecules necessary for avoiding T cell anergy. DC's were isolated from human peripheral blood using IL-4 and GM-CSF. MUC1 was transfectd into DC's by lipofection. After treatment of the cells with a glycosylation inhibitor, they exposed high levels of the relevant mucin epitopes. Autologous DC's were used to repetetively stimulate T cells of healthy donors. Proliferation assays were performed using MUC1 transfected DC's treated with and without glycosylation inhibitor, DC's pulsed with mucin peptides and mucin peptides from different length alone as stimulators. Their capability to stimulate resting and activated T cells was evaluated. Tumor specific cytotoxic T cells were established using MUC1 transfected DC's treated with the glycosylation inhibitor. They lyse different mucin expressing tumor cell lines independently from the HLA-phenotype. MUC1 cDNA transfected dendritic cells exposing the relevant epitopes will be used as tumor vaccine in a clinical phase I trial.

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ADOPTIVE IMMUNOTHERAPY (TIL + IL-2) AFTER METASTASECTOMY IN 34 PATIENTS WITH MELANOMA, COLORECTAL AND RENAL CARCINOMA

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Twelve advanced patients (7 melanoma, 4 colorectal carcinoma, 1 kidney carcinoma) and 22 patients in an adjuvant setting after metastasectomy (11 melanoma, 10 colorectal carcinoma, 1 renal cancer), were treated. No objective response was observed in advanced patients: all patients progressed after a median of 1,5 months (range 0-8), while median survival was 8 months (range 3-20+); 13 patients from the second group are still disease free after a median of 21+ months (range 7+-45+). The remaining 9 patients relapsed after a median of 5 months (range 3-18). Toxicity was moderate as clinical and hepatic/renal function parameters were used to assess the need for dose reductions. Cytolytic activity was evaluated was evaluated on autologous tumor tissue in only 2 melanoma cases. In all the other patients evaluation was made on allogenic tumors and stabilized cell lines. Perforin levels in TIL measured at the end of culture were generally high or very high. Cytokines levels were measured on the surnatant at the end of culture. The estreme variability in the results obtained prevents any correlations from being made with clinical data.
Tzeta chain and $P56^{lck}$ were histologically assessed on the resected tissue from which TIL were cultivated. Virtually, none of the former were found, and there was a complete absence of the latter which concurs with data reported in the literature. The same immunocytochemical analysis was carried out on TIL at the end of culture. This time a almost complete restoral of both functions was seen especially in patients who asre still DF.
The study is on-ging and it has been decided to focus on DF patients after metastasectomy in order to increase the number and possibility of clinical and histological correlations.

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THE ROLE OF IMMUNOMODULATION ON ANTI-TUMOUR ACTIVITY OF HEXADECYLPHOSPHOCHOLINE

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Hexadecylphosphocholine (HPC) is active against experimental and clinical breast cancer *in vivo*, however the mechanisms of its action are unclear. The aims of this study were to investigate the role of immunomodulation on anti-tumour activity of HPC *in vivo* and *in vitro*. In the *in vivo* studies, MT-1 tumours transplanted subcutaneously to mammary fat pads of 12 week old NCR/nu mice were allowed to grow for approximately 22days. HPC, in 10%Tween 80/saline was administered orally (50mg/kg/day) for 5days. 10% Tween 80/saline alone was administered to control mice at the same time. One week after the end of treatment, mice were killed, secondary immune tissues and tumours removed for histology, immunocytochemistry and RT-PCR analysis. Comparison of lymph node sections from control mice with those from HPC treated mice showed a significant increase in the number of immune cells following treatment. Immunocytochemistry showed a significant increase ($p<0.05$) in macrophages, T cells and T cytotoxic cells after treatment with HPC. Treatment resulted in tumour lysis accompanied by significant infiltration of macrophages and lymphocytes. In addition, HPC treated tumours showed a significant increase ($p<0.005$) in endothelial cells stained by anti-CD31 and anti-vWF antibodies. In order to investigate the functional activity of these infiltrating cells, cytokine mRNA expression was assessed by RT-PCR. mRNA for IFNγ, IL-1β and TNFα was detected in HPC treated tumours. In *in vitro* studies, splenocytes from nude mice were incubated with medium alone or different concentrations of HPC for 3, 24, 48 and 120h. The ELISA assay was determined to detect TNFα production. Significant TNFα production was observed in samples incubated for 48h at 61.3μM. The effects of HPC on MHC expression on MT-1 cells were also investigated, however, no increase on MHC expression was observed. These effects did not occur with standard cytotoxic therapy, so the results suggest that immunomodulation may play a role in the anti-tumour effects of HPC.

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MULTIDRUG RESISTANT MALIGNANT MELANOMA WITH INTRACRANIAL METASTASIS RESPONDING TO IMMUNOTHERAPY

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Malignant melanoma (MM) is well known for its poor response to chemotherapy, radiotherapy, and its remarkable susceptibility to interleukin-2 (IL-2) based immunotherapies. Drug efflux pump such as p-glycoprotein (P-gp), or drug detoxyfying mechanisms e.g. glutathion S-transferase-pi (GST) are some of the multidrug resistance (MDR) mechanisms in MM. MM with brain metastatis has 4-5 months life expectancy from metastasis to death. **Here we report the first $P\text{-gp}^+$ MDR MM with brain metastasis in the literature demonstrating a remarkable response to IL-2, interferon-alpha (IFN), 5-fluorouracil (5FU) regiment.**
A 41-year old man was admitted with headache, and gait disturbances. Inoperable brain lesions were detected, and biopsies revealed MM. Cisplatin, carmustine, dacarbazine, tamoxifen (CCDT) together with radiotherapy was administered, and partial response with almost complete disappearance of initial symptoms was achieved. However, after the third CCDT course, he had confusion and various neurological signs. Dimensions of his cranial lesions were dramatically increased and his dermal lesion recurred. Biopsy from this lesion revealed that MM cells were $P\text{-gp}^+$, but GST-, $P\text{-gp}^+$ MDR cells were susceptible to IL-2 activated killer cells in previous studies with various cell lines. IL-2, IFN, 5FU was administered and remarkable decline in the dimensions of intracranial lesions was observed with almost total disappearance of symptoms. He is well and capable of doing work for 14^+ months. Dermal lesions haven't recurred during immunotherapy. This case suggests that $P\text{-gp}^+$ MDR MM is a good candidate for IL-2 based therapies.