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**Sequential stimulation of dendritic cells with cytokines followed by CD40L enhances their T cell stimulatory capacity**

**Background:** Tumor vaccines based on dendritic cells (DC) aim at inducing a T-cell response against tumor antigens. To obtain an immunostimulatory phenotype, DC need an activation signal mediated by inflammatory substances, microbial products or CD40-ligation via activated T-cells. We examined the influence of the activation signal on DC phenotype, survival, IL-12 secretion and T-cell activation. **Methods:** DC were generated by culturing adherent MNC in the presence of GM-CSF and IL-4. DC were pulsed with antigens from pancreatic carcinoma cells, activated with cytokines with or without CD40-Ligand (CD40L) and cocultured with autologous T-cells. **Results:** Only DC activated with CD40L in combination with cytokines produced high amounts of IL-12p70, upregulated the costimulatory molecules CD80 and CD86 as well as the chemokine receptor CCR7, which is essential for the homing of DC to the lymph node. 13% of cocultured T-helper cells and 18% of CTL expressed the activation marker CD69, vs. 5% and 4%, respectively, if DC were activated by cytokines or CD40L only. Compared to simultaneous stimulation, sequential activation of DC with cytokines followed by CD40L enhanced DC survival and expression of costimulatory molecules. CTL are currently tested for their cytotoxic activity against pancreatic carcinoma cells. **Conclusion:** The sequence of providing activation signals to DC might influence the potency of DC-based tumor vaccines.

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**MODULATION IN PHENOTYPE AND FUNCTION OF DENDRITIC CELLS IN EARLY BREAST CANCER.**

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**Rationale:** Previous observations from our laboratory have shown deficiencies in antigen presentation and other functionality of monocytes derived from patients with early breast cancer (EBC). We have now expanded our investigations to dendritic cells (DCs) which have been only insufficiently studied in patients with EBC.

**Objective:** In order to analyze their phenotype, DCs derived from patients with EBC were analysed for the expression of DC-specific antigens CD1a, CD83, CD80, CD86, CD54 and CD14. The functional capacity of antigen presentation by DCs was evaluated by a T cell proliferation assay using tetanus toxoid (TT) as antigen.

**Methods:** Peripheral blood from 36 patients with EBC and from 26 healthy age-matched female controls was drawn and prepared for *ex vivo* DC generation by two standard procedures consisting of the use of either granulocyte/macrophage-colony stimulating factor (GM-CSF) together with interleukin 4 (IL4) and tumour necrosis factor (TNF)- $\alpha$  or GM-CSF with IL4 alone. DC phenotype was examined by flow cytometry. T cell proliferation in response to TT-pulsed DCs was measured by

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**Hsp70-peptide activated autologous NK cells in the immunotherapy of cancer – a clinical pilot study**

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As previously shown an incubation of NK cells with a 14-mer Hsp70-peptide stimulates both the proliferation and cytolytic activity against Hsp70 positive tumors, *in vitro*. An immunoreconstitution of tumor-bearing mice with Hsp70-peptide activated NK cells results in tumor regression. Before starting the clinical trial about 500 different tumor biopsies and bone marrow aspirates of leukemic patients have been screened for Hsp70 membrane expression. Especially lung, colorectal, pancreas cancer and leukemic blasts have been defined as Hsp70 positive. Therefore, patients with these tumors were included in our first clinical trial. Peripheral blood mononuclear cells (PBMC) of Hsp70 positive cancer patients were isolated by leukapheresis followed by Ficoll separation. Then PBMC were transferred into 250ml tissue culture bags (Cellgenix) and incubated for 4 days with Hsp70-peptide (cGMP-grade) plus low dose IL-2 (100 IU/ml) in serumfree X-Vivo 20 medium (GMP-grade). Following two washing steps the activated cells were reinfused into the patient on day 4. So far 6 patients suffering from solid tumors and 4 leukemic patients have been treated. So far, none of the patients showed any negative side effects. In *in vitro* assays we demonstrate for all patients an activation of NK cells after treatment by the determination of cell surface markers (FACS) and in functional assays (standard Cr-51 release assays).