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Detection of tumor cells in the bone marrow of breast cancer patients by cytokeratin 19 - reverse-transcriptase - polymerase chain reaction (RT-PCR)

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Aim of the study: To evaluate the individual risk of breast cancer patients at the time of primary diagnosis and to establish a risk adapted adjuvant therapy, the detection of tumor cells in the bone marrow is performed by immunocytochemical staining for cytokeratin 18 (CK 18). In former studies tumor cell detection was correlated with lower overall survival rates due to early metastasis. Methodically, antibody-cross reaction with normal bone marrow cells is a substantial problem. Tumor cells are detected at a rate of $1/10^4$ - 10^5 normal cells. The aim of the study was to increase specifity and sensitiviy of tumor cell detection by using the PCR method for cytokeratin 19 (CK 19) which enhances the detection of tumor cells up to $1/10^7$ normal cells. Both methods are compared.
Methods: The bone marrow aspirates of 35 patients were purified and separated by Ficoll gradient centrifugation, stained immunocytochemically and investigated by RT-PCR. For the PCR procedure we used two primerpairs with 35 cycles of polymerisation each time. The specimens were evaluated after incubation with a P_{32} - probe. As negative controls we used water and normal bone marrow aspirates. Cytokeratin19 positive malignant breast tumor and the tumor cell line T47D served as positive controls.
Results: Both methods were performed in 35 patients. Up to now, the results of 10 patients have been evaluated. CK18- and CK19-detection correlated closely in 6 patients (3 negative and 3 positive). In 4 patients we could demonstrate positive results with the PCR method while the immunocytochemical staining was negative. In no case a negative PCR was combined with positive immunocytochemical tumor cell detection.
Conclusion: In this pilot study the PCR method seems to be more specific and sensitive for the detection of tumor cells in the bone marrow of breast cancer patients compared to the immunocytochemical staining procedure. Further studies with a greater number of patients are needed. In addition, the correlation to other prognostic factors, to the clinical outcome and therapeutic consequences have to be investigated.

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CELLULAR IMMUNOTHERAPY OF ACUTE LEUKEMIA WITH NATURAL KILLER CELLS IN AN ANIMAL MODEL. M. Zeis, L. Uharek, B. Glass, J. Steinmann, H. Löffler, W. Mueller-Ruchholtz and W. Gassmann. Department of Internal Medicine II and Institute of Immunology, University of Kiel

The transfer of allogeneic bone marrow cells provides a benefical antileukemic effect, a phenomenon called the graft-versus-leukemia (GvL) effect. We previously demonstrated GvL effects independent from GvHD and correlated to NK activity of the BM donors in a murine model. In this study, we investigated the antileukemic potential of adoptively transferred enriched NK cells from both syngeneic (Balb/c, H-2^d) and allogeneic (C57B6, H-2^b) origin. Balb/c mice were given a lethal dose of A20 (H-2^d, B cell leukemia) cells 2 days prior to lethal (7.5 Gy) total body irradiation (TBI) and transplantation of either syngeneic or allogeneic Thy1.2 pretreated BM cells. In different experimental groups either syngeneic or allogeneic enriched NK-cells were given shortly after BMT. Some groups received NK cells ex-vivo incubated with IL-2. Injection of A20 leukemia led to death after a median of 30 days. A lethal dose of TBI followed by either syngeneic or allogeneic Thy1.2 depleted BMT resulted in a slight antileukemic effect. The adoptive transfer of syngeneic enriched NK-cells given at time of BMT exerted a significant GvL effect. However, infusion of allogeneic (H-2^b) MHC-mismatched enriched NK cells resulted in a stronger GvL effect compared to syngeneic NK-cells. Ex-vivo incubation of NK cells with IL-2 did not further enhance GvL activity. These results clearly demonstrate that immunotherapeutic transfer of NK cells can induce strong antileukemic activity without clinical overt GvHD.

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INTERFERON-ALPHA AND VINBLASTINE VERSUS MEDROXY-PROGESTERONACETAT IN THE TREATMENT OF METASTATIC RENAL CELL CARCINOMA

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Objectives. Since the beginning of the eighties, when gene technology provided sufficient amounts of cytokines, numerous phase II studies in metastatic renal cell carcinoma were carried out mostly with Interferon-α (IFN-α) and Interleukin-2 (IL-2). So far no randomized prospective trials including untreated control groups have been reported. The authors present a prospective study comparing IFN-α and vinblastine (VBL) versus Medroxyprogesteronacetat (MPA).

Methods. Immunochemotherapy schedule consists of IFN-α 8 million U/day subcutaneously for 3 days per week and VLB 0.1 mg/kg bodyweight intravenously at 3 weeks intervals. MPA was administrated intramusculary at a dosage of 500 mg per week.The response rates, toxicities and actuarial overall survival were analyzed.

Results. The overall response rate in 41 patients receiving IFN-α/VLB treatment was 22% (95% confidence interval 9-35%). 4 patients reached a complete and 5 patients a partial remission. No remissions were observed in 35 patients of the control group. A statistically significant survival benefit for the IFN-α/VLB-group could not be demonstrated. Excluding fever, mild to moderate toxicities were observed. About one third of patients refused the proposed schedule due to general malaise and fatigue.

Conclusions. A survival benefit or a favorable outcome of patients with metastatic renal cell carcinoma, treated with IFN-α/VLB could not be demonstrated. As judged from this analysis IFN-α/VLB therapy does not sufficiently meet the requirements of a palliative treatment of renal cell carcinoma.

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Gene Modification of Primary Tumor Cells for Active Immunotherapy of Human Breast and Ovarian Cancer.

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We have previously shown that cationic liposomes facilitate adeno-associated virus (AAV) plasmid transfections of primary and cultured cell types. To test the clinical feasibility of using genetically modified tumor vaccines for the treatment of breast and ovarian cancers, we have constructed an expression plasmid pMP6IL2 and investigated the use of liposome mediated gene delivery into primary, uncultured human breast and ovarian tumor cells to produce IL2 secreting tumor cells. We have demonstrated significant levels of IL2 expression in tumor cell lines and primary breast and ovarian tumor cells using this AAV-based expression plasmid complexed to cationic liposomes. Transfections with the non-AAV plasmid containing the identical expression cassette as the AAV-plasmid induced IL2 expression in the tumor cell line, but failed to produce IL2 in primary tumor cells. Significant levels of IL2 were induced with the AAV plasmid regardless of liposome compositions used for transfection. The transfected breast cell line and primary tumor cells were able to express the transgene product for up to 28 days post lethal radiation. The transfection efficiency was comparable for both the tumor cell line and primary tumor cells and ranged from 20-50% as assessed by intracellular IL2 staining. In primary tumor cell preparations, 40% of the tumor cells expressed the transgene as assessed by immunostaining for IL2. IL2 secreted as a result of gene expression was biologically active and capable of causing proliferation of an IL2 dependent cell line. The ability to efficiently express transgenes in freshly isolated, non-dividing, uncultured tumor cells may potentiate active immunotherapy strategies for gene based cancer treatment.