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The use of genetically modified tumor cells as a vaccine in patients with cancer: The definition of intermediate end-points.

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There are many studies currently exploring the role of genetically modified cells in patients with cancer. Since these are Phase I studies, tumor response is not a primary end point. It has been shown that murine tumour cells transduced with the GM-CSF gene generate a potent anti-tumour immune response when used as a vaccine in mice (Dranoff et al P.N.A.S. 1993 90: 3538-43).

In our ongoing Phase 1 study (in collaboration with Somatix Therapy Corporation, Alameda, California), patients {pt} with metastatic malignant melanoma are vaccinated with autologous, irradiated tumour cells transduced with the gene for huGM-CSF using the MFG-S retroviral vector. 28 pt are randomised to receive either 5 x 10^6 or $5x10^7$ cells per vaccination (secreting 40-1000 ng GM-CSF/10^6 cells/24 hr). For each patient, three vaccinations are given at 21 day intervals. Half the vaccine is given subcutaneous and half intradermally. Injections to measure DTH responses are given simultaneously at a distant, separate site using $0.5x10^6$ autologous non-transduced tumour cells. The end points of this Phase 1 study are to establish toxicity and safety of this approach, and to determine the immuno-modulatory effects.

Unlike the generally accepted definitions for toxicity and response, there are no commonly accepted criteria for the measurement of an immune response. It is necessary to establish these for protocols involving genetic modifications when the aim of therapy is to improve recognition of the tumor by the immune system. Consensus is required regarding the assays and measurements used for the determination of a cytotoxic T cell response or a delayed type hypersensitivity.

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Interleukin-2 transfected melanoma cells for tumor vaccination. - From animal models to clinical trials -

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We have established a preclinical model for the immunological rejection of melanoma cells in a syngeneic host. DBA/2 mice were immunized twice with irradiated, IL-2 secreting M-3 melanoma cells. Cells were transfected with the murine IL-2 cDNA by using the adenovirus-enhanced transferrinfection method. Upon challenge with native tumor cells, only vaccinated animals were protected effectively from tumor development. In an attempt to explore the cellular mechanisms responsible for IL-2 induced tumor rejection in this model, we identified cells mediating protection after challenge with tumorigenic M-3 cells. Immunohistological characterization of the challenge site showed a substantial increase in both CD4- and CD8-positive T cells in animals immunized with IL-2. In order to demonstrate that T lymphocytes confer systemic immunity, splenic T cells from immunized animals were enriched for CD4+/CD8+ subpopulations, respectively. T cells were mixed with parental tumor cells and injected into naive mice. As a result, only animals receiving both T cell subpopulations were protected effectively from tumor development. In addition, no protection could be induced in athymic nude mice showing that T cells are required for anti-tumor immunity.

Since there is some controversy about the site and mode of T cell activation after IL-2 tumor vaccination, we examined the immunological effects at the immunization site as well as downstream events. We could show, that macrophages, granulocytes and NK-cells infiltrate the vaccination site early after injection and eliminate the inoculum within 48h. We could not find T cells at the vaccination site, which argues against the concept that T cell priming by the IL-2 secreting cancer cells occurs directly at that location. However, RT-PCR revealed transcripts indicative of T-cell activation in the draining lymph nodes of mice immunized with the IL-2 secreting vaccine, but not in mice vaccinated with untransfected, irradiated M-3 cells. We therefore propose that the antigen-presenting cells (APCs), which invade the vaccination site, process tumor-derived antigens and subsequently initiate priming of tumor-specific cytotoxic T-lymphocytes in lymphoid organs.

Adenovirus enhanced transferrinfection is an very efficient gene transfer method resulting in cytokine levels produced by transfected melanomas which are in general much higher than those achieved with other procedures. We observed that after vaccination with M-3 cells expressing IL-2 levels as high as 50.000 units/10^6 cells/24 hours and higher, animals were no longer protected. Mechanisms leading to the IL-2 dose-dependent failure from tumor development will be discussed.

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STRATEGIES FOR THE DEVELOPMENT OF A TUMOR VACCINE FOR NON-HODGKIN'S LYMPHOMA

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Patients with relapsed high and intermediate grade non-Hodgkin's lymphoma (NHL) have a poor prognosis. Therefore, these patients are often treated with high-dose chemotherapy followed by autologous bone marrow or stem cell transplantation. Nevertheless, the majority of the patients undergoes uncurable relapses which frequently arise from a very small number of residual tumor cells. Therefore, we aim to develop an adjuvant tumor vaccine for NHL by transfer of immunomodulatory genes in lymphoma cells. For this purpose, we have developed an effcient vector system based on adeno-associated virus (AAV). With an optimized packaging system, recombinant AAV (rAAV) titers up to 10^{11} particles/ml can be obtained which allow an efficient transfer of different genes in lymphoma cells within 24 to 48 hours. This recombinant AAV vector was used to transduce the genes of the T-cell costimulatory molecules, B7-1 (CD80) and B7-2 (CD86), as well as the genes of ß-galactosidase (ß-gal) and neomycin resistance (neo^R) into lymphoma cell lines (RPMI-8226 and LP-1). This allowed the expression of B7-1 or B7-2 in up to 80% of the lymphoma cells 48 hours after infection with rAAV. Lymphoma cells transduced with B7-1, B7-2, or ß-gal and untransduced cells were used to stimulate T-lymphocytes of healthy donors *in vitro*. B7-1 or B7-2 transduced lymphoma cells induced an up to sixfold higher T-cell response in proliferation assays than ß-gal transduced or untransduced lymphoma cells. Interestingly, the T-cell response to the B7-2 positive lymphoma cell line RPMI-8226 was strongly increased by B7-2 gene transfer, suggesting that endogenous B7-2 expression of these cells was not sufficient to induce a full immune response. Taken together, the results show that rAAV-mediated gene transfer of T-cell costimulatory molecules like B7-1 and B7-2 to lymphoma cells is able to augment the T-cell response. Additional T-cell (co)stimulatory molecules and lymphoma-associated antigens which are tested for this approach will be discussed.

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PLASMID VACCINATION FOR B-CELL LYMPHOMA

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Treatment of cancer with vaccines is an attractive prospect and the idiotypic immunoglobulin of B-cell lymphomas is a suitable tumour specific target. Animal models suggest this approach can be effective but making vaccines on an individual basis is time consuming and difficult. We have investigated the use of the polymerase chain reaction (PCR) to identify the tumour idiotype, followed by a novel method of idiotypic vaccination based on the direct injection of a recombinant vector in the form of plasmid DNA.

The use of PCR to amplify and clone genes from routine clinical biopsies followed by DNA sequencing enables us to rapidly identify tumour derived V-genes from lymph node biopsies of patients with B-cell lymphoma. Both the VH and VL genes have been identified in 11/13 pateints. For vaccination we use an expression vector with a retroviral promoter (rous sarcoma virus) that expresses the functional immunoglobulin, in the form of a single chain Fv (scFv) fused to short peptides. Intramuscular injection of plasmid DNA (10-100µg) in saline results in the generation of specific anti-idiotypic antibody and proliferative T-cell responses. There was no apparent toxicity and no detectable spread of the DNA from the injection site. The DNA persists at the injection site for up to six weeks. Purification of plasmid DNA for clincal trials is relatively straightforward and currently a phase I clinical trial is underway in ten patients with relapsed/persistent disease after conventional therapy.

Second generation vectors, which co-express cytokines such as IL2 or GM-CSF, are being tested in animal models and can generate enhanced anti-idiotypic responses. These may thus be advantageous but prolonged expression from these vectors can result in immune complex formation. This and safety considerations suggest that control of expression may be desirable with these modified vectors.

These techniques should allow the development of effective and practical anti-idiotype vaccination and may also be valuable to immunise against other tumour antigens consisting of self- or mutated self-proteins.

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