

S25
IMMUNO-GENE THERAPY FOR TUMORS – WHAT IS NEEDED FOR SUCCESS

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Immune-related genes have been used to induce an anti-tumor response which destroys not only the tumor in which the transgene is expressed but also distal tumor deposits, eg. occult metastases.

After studying nearly 100 transfectants in four different strains of mice using over 20 different tumor cell lines we have come to the following broad conclusions: 1). Strongest anti-tumor immune reactivity is generated using IL-12, GM-CSF, IL-4, IL-2 and/or B7-1/2) Therapy needs to be initiated early in the disease and needs to be continuous- the only therapy that can induce regression of late, established tumor is apoptosis-induction followed by a strong CD40 signal 3) Failure of host anti-tumor immunity is not due to "ignorance" of tumor antigens but due to the development of ineffective/inappropriate antigen presentation.

Clinical trials using a vaccinia-IL2 construct intratumorally showed that this approach is safe but dramatic tumour shrinkage is yet to be observed. We have studied the capacity of dendritic cells (DCs) to act as vehicles for this vector. VV-IL-2 constructs infect human DCs efficiently but induce the DCs to release IL-4 rather than IL-12 ie. a potentially dangerous "tolerising" pattern of cytokines. In our murine mesothelioma model VV-IL-2 delivered intratumorally induced slowing or regression of tumor. We are currently evaluating CMV as a vector.

Tumor immuno-gene therapy must be based on a solid knowledge of how tumors engage with host anti-tumor immune cells.

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POTENTIAL OF IMMUNOTHERAPY IN PREVENTING TUMOR RELAPSE/METASTASIS OF HUMAN CERVICAL CANCER: A PILOT STUDY

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Cervical cancer is well suited for intra-tumoral gene immunotherapy as the primary tumour is easily accessible for direct injection of DNA-liposome complexes. The usual time lapse between diagnosis of cancer and initiation of radiotherapy is an opportune window period during which gene immunotherapy can be instituted. Enhanced cytotoxic immune response may reduce the incidence of tumour recurrence locally and in distant sites.

Ten patients were recruited, eight with squamous cell carcinoma and two with adenocarcinoma. All patients received four injections of HLA-liposome complexes at weekly intervals. Seven patients were given HLA-A2 and three were given HLA-B13. No adverse reactions from the patients were observed. No changes were detected in the dimensions of the tumours during the period under gene therapy, and all the patients received the standard external beam and intracavitary radiotherapy. Nine patients showed complete response to radiotherapy. One patient with stage 2B adenocarcinoma showed a partial regression of the tumour. With a median follow up of 46 (18-60) months, two local recurrences of the tumours were detected and the partial responder died of progressive disease despite cytotoxic chemotherapy. Gene immunotherapy offers an attractive approach to enhance host anti-tumour immune response. Our approach with allogeneic MHC DNA (HLA-A2 and HLA-B13) and liposome vectors is safe and has been shown to be able to elicit good anti-tumour activity in some cervical cancer patients. This approach of gene therapy overcomes the technical difficulty of low efficiency of tumour cell transfection with candidate genes *in vivo* since only a small proportion of tumour cell modification is sufficient to elicit the desired immune response. A randomised controlled trial is warranted to investigate if gene immunotherapy in combination with conventional therapy will prevent tumour relapse or metastasis.

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IMMUNO-GENE THERAPY TRIALS USING TUMOUR mRNA TRANSFECTED DENDRITIC CELLS

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In two ongoing phase I/II trials, patients with hormone resistant prostate cancer and stage IV melanoma are vaccinated with autologous dendritic cells (DC) that have been transfected either with mRNA derived from standard prostate cancer cell lines or from each patient's own tumour (melanoma). Transfection by electroporation results in high expression of proteins encoded by the mRNA. DC are produced under GMP conditions in a closed system with IL4 and GM-CSF for 5 days. After transfection, immature DC are matured for 2 days and the resulting vaccine is frozen in aliquots. Each patient receives 4 weekly vaccines. Until now 13 patients have received the vaccine intradermally and 21 intranodally (ultrasound guided). No serious adverse effects have been seen to date. In 12/15 prostate and 11/19 melanoma patients, an immune response was obtained based on *in vitro* immunoassays and/or a DTH response. T cell clones specific for mRNA transfected DC could be obtained from several patients and some of these were shown to kill HLA a matched prostate cancer cell line used for mRNA production. Mixed tumor responses, with disappearance of metastatic lymph node and subcutaneous tumors and development of vitiligo were observed in melanoma patients. In the prostate cancer group, immune responses were associated with stable or reduced levels of PSA. We conclude that the strategy of vaccinating with mRNA transfected DC functions to elicit cellular immune responses, and that such responses may be associated with a clinical benefit.

S28
DEVELOPMENT OF A NOVEL NON-VIRAL VECTOR SYSTEM FOR CANCER TREATMENT

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To develop highly efficient and minimally invasive vector system, we have attempted to design a delivery system using non-infectious viral particles with inherent viral properties. Along these lines, we first developed HVJ-liposomes, and then HVJ envelope vector (HVJ-E) using inactivated HVJ (hemagglutinating virus of Japan; Sendai virus). With the fusion activity of HVJ, HVJ-E introduced plasmid DNA efficiently both *in vitro* and *in vivo* without liposomes. HVJ-E is also very effective for the delivery of drugs such as antibody, peptide, siRNA and anti-cancer drugs.

Using HVJ-E containing plasmid DNA encoding TAAs, murine bone marrow derived DCs were transfected *ex vivo*. Transfected DCs expressing TAAs gp100 and TRP-2 were shown to stimulate autologous T-cells *in vitro* and elicit TAA-specific immune responses in syngeneic mice. Injection of HVJ-E-transfected DCs expressing TAAs, cured the mice from lethal challenge with BL6 melanoma cells.

Another critical issue in gene therapy is the enhancement of delivered gene in target cells. Based on the study of chromatin remodeling, we succeeded in enhancing transgene expression using a histone deacetylase inhibitor, FR901228. Luciferase gene expression was 50 – 5000 fold augmented by the drug in both cultured cells and tumor mass in mice. With the aid of the drug, suicide gene therapy of tumor-bearing mice was very effective and complete response was observed in the mice.