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Peptide loaded human autologous dendritic cells (DC's) as a potential anti-cancer vaccine

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Rationale: DCs are powerful antigen presenting cells which, when loaded with tumour associated antigen peptides (TAAPs) can prime specific cytotoxic T cells (CTLs) to exert powerful anti tumour effects against murine tumours expressing the antigen (Mayodoma et al Nature Med 1: 1297, 1996). Such DCs may be prepared from human peripheral blood mononuclear cells (PBMCs) using GMCSF & IL4, and when antigen primed represent a potential specific anti-cancer vaccine.

Aims of Study: To characterise DCs prepared from PBMCs of normal volunteers and cancer patients for their ability to induce in vitro autologous CTL response against AAPs derived from HPV16E7 and HER-2 antigens, as a feasibility study prior to clinical application.

Methods: Autologous DCs were cultured using method of Romani et al (J Exp Med 180: 183, 1995) from 8 human volunteers and 4 cervical cancer patients and their yield and morphological and FACS characteristics determined at 8 days. These were loaded with haplotype specific TAAPs – HPV16E7 (YMLDLOPETT) and HER-2/neu (KIFGSLAFL) respectively. The flu peptide (SRYWAIRTR) was used as a positive control. PBMCs were primed with these DCs and tested for their CTL activity against peptide loaded autologous EBV transformed B cells.

Results: Cells with morphological and immunophenotypic features of DCs were grown from all individuals with yields ranging between 4.0–10.8 $\times$ 10^5/20 mls blood. All DC preparations also showed uniformly strong positivity for Class II, CD80 (B7.1) C54 (ICAM1) & CD86 (B7.2). CDla was variable expressed in the volunteers (22–25% cells) but consistently negative in the cancer patients. Specific MHC class 1 restricted CTL responses have been demonstrated in 3 normal HLA-2 volunteers to all 3 peptides and to HPV16E7 in 1 cervical cancer patient tested to date.

Conclusion: It is feasible to prepare functionally active DCs from cervical cancer patients with the same efficiency as the normal volunteers and induce specific MHC class 1 restricted responses to TAAPs. DCs primed with TAAPs therefore have a potential in anti-cancer immunotherapy.

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Immunization of melanoma patients with peptide-pulsed dendritic cells

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Purpose: Cytotoxic T lymphocytes (CTLs) attack melanoma cells in a MHC restricted and tumor antigen specific manner. Several melanoma-associated antigens were identified recently. Such antigens are ideal candidates for any kind of vaccination approach in melanoma. Dendritic cells (DC) are effective in the induction of primary T cell responses making DC an ideal candidate for the induction of anti-tumor immunity in melanoma.

Methods: DC were generated from peripheral blood using GM-CSF and IL-4 over 8 days and pulsed with a cocktail of peptides known to be recognized by CTLs depending on the patient's HLA haplotype. 5 $\times$ 10^6 DC were weekly injected into inguinal lymph nodes over 1 months. Immunizations were repeaed in monthly intervals up to 10 injections. In parallel, clinical response was monitored as well as DTH reactivity, T-cell reactivity to peptides by ELISPOT assay and immunhistological expression of tumor antigens.

Results: Vaccination was well tolerated by all patients. Some patients developed a positive DTH reactivity to peptide- pulsed DC. So far, clinical responses were evident in 4 out of 9 evaluable patients (2CR, 2PR). Further immunological evaluation is in progress.

Conclusion: Vaccination with autologous DC generated from peripheral blood seems to be a new, safe and promising approach in the treatment of metastatic melanoma.

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Six years experience using inhalatory interleukin-2 in pulmonary metastatic renal cell carcinoma

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Purpose: Evaluate effective immunotherapy with high quality of life

Methods: 116 patients use inhalatory IL-2 application, a non-toxic, effective treatment for patients with progressive pulmonary metastases of renal cell carcinoma (RCC). In different protocols three different IL-2 preparations of IL-2 were used. All protocols had in common a high-dose inhalatory IL-2 application either exclusive (11%), in addition with low dose systemic IL-2 (33%), or low dose systemic IL-2 and interferon-a (56%).

Results: Maximum toxicity per total treatment time (median treatment time 7.2 months) was mild (WHO grade III 16%), mainly cough. Explicitly, pulmonary disease was influenced. Progressive pulmonary metastases responded in 15% for a median of 15.5 months (4.1–33) and were stabilised in 55% for a median of 6.6 months (3–51.7). Overall response rate was 15%, stable disease 50% and progressive disease 35%. Median overall response duration was 9.6 months. Median achieved survival was 11.8 months (1.7–68.8) while expected survival according to risk analysis was 5.3 months.

Conclusion: Inhalatory IL-2 prevents progress of pulmonary metastases effectively in 70% of patients. Low toxicity allows outpatient therapy, employment and good quality of life.

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E1B gene defective adenoviruses as an antitumor treatment in human cancer

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Purpose: The E1A gene of adenovirus 5 induces marked sensitivity to chemotherapy and radiotherapy and exerts an antitumor effect *in vivo* that is counteracted by the E1B gene. The purpose of this study is to determine the therapeutic potential of E1B-defective adenoviral vectors, owing to the effect of the E1A gene.

Methods: The human tumor cell lines used were: HeLa, Saos-2, A431, HT29 and MCF7; a group of uveal melanomas including MKTBR, SP6 and OCM1 tumor cell lines; and a nontumoral melanocytic cell line, UW3. Wild-type (WT) 300, adenovirus type 5 and two deleted mutants (from Dr. Ginsberg and Dr. Shenk) were employed: Ad dl 118 (with the E1B region deleted but E1A intact) and Ad dl 312 (with E1A deleted but E1B intact). For drug treatment, cisplatin (Bristol-Myers) was used at a dose of 1 μg/ml. In irradiation assays, cells were gamma-irradiated with a Co-60 source. Cell density was evaluated by the crystal violet method.

Results: The Ad dl 118 mutant produced marked but variable lethality in all infected cells. Viability at 70 h postinfection was 10%–110% for the Ad dl 118 mutant vs 100–389% for the WT form, 97%–619% for the Ad dl312 mutant and 180%–528% for uninfected controls. In melanic cell lines, after infection at 200 PFU/cell and chemotherapy or radiotherapy, cells infected previously with Ad dl 118 mutant were more sensitive to treatment.

Conclusions: The results suggest that infection with E1B-defective adenoviruses expressing E1A produces a marked cytotoxic and antitumor effect. Thus, the use of these vectors is proposed as a novel antitumor approach in human anticancer therapy.

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The E1a Gene as antitumor agent: Trials in murine experimental models

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Purpose: The adenovirus E1a gene is a potent inducer of chemosensitivity and radiosensitivity by p53-independent mechanisms in different cell models. We evaluate the *in vitro* and *in vivo* effects of the E1a gene.

Methods: HaCa4 and MSC11 A5 (moderately differentiated and sarcomatoid epidermoid carcinomas, respectively) were used. These cell lines present several genetic alterations including H-ras or p53 mutations. The expression vector chosen was the MD-E1, a retrovirus that encodes for the 13S transcript of E1a. The results of dose/response trials with cisplatin (at doses of 1 to 10 mg/ml) and radiation trials (from 1 to 8 Gy) were quantified

using the crystal violet method. Tumorigenicity studies were performed in athymia mice. Intratumor coinjection and injection of tumor cells (HaCa4, MSC11A5) and MD-E1a retrovirus-producing cells were also performed.

Results: The E1a gene induced marked sensitivity to cisplatin and to radiation in both cell models (80% greater than in control cells lacking E1a expression). *In vivo* assays showed that constitutive E1a expression increased latency and decreased tumorigenicity. The results with coinjection of MD-E1a virus-producing cells were identical to those obtained *in vitro* using transfected lines. Moreover, intratumor injection of producer cells partially blocked the growth of tumors generated by MSC11A5 cells.

Conclusions: The E1a gene induces chemosensitivity and radiosensitivity in epidermoid carcinoma cell lines regardless of the p53 status. Injection of E1a-producer cells may block tumorigenicity and results in a new approach for gene therapy in cancer.

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Employment of the mdr1 promoter for a conditionally active retroviral vector system in cancer gene therapy

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Purpose: Inducible vectors are attractive tools for the conditional expression of therapeutic genes in cancer gene therapy. Earlier studies demonstrated that the promoter of the multidrug resistance gene (mdr1) harbors responsive elements that are inducible by MDR-associated drugs. This points to the applicability of the mdr1 promoter for the construction of drug-inducible vectors. We linked an mdr1 promoter element to the human TNFα gene in a retroviral vector to evaluate expression efficacy and drug-inducibility of this system.

Methods: The retroviral constructs were transduced into MCF-7 human mammary carcinoma and HCT116 human colon carcinoma cells. For the induction experiments transduced cells were treated with doxorubicin, vincristine, VP-16 and taxol. The expression and induction studies were performed by using RT-PCR and TNFα-specific ELISA.

Results: Transduced MCF-7 and HCT116 cells showed measurable basal expression of TNFα. Treatment of the cells with the MDR-associated drugs led to a 2–3 fold increase in TNFα mRNA followed by an 3–13 fold increase in TNFα secretion. This induction was drug-concentration dependent.

Conclusion: The studies have shown that the mdr1 promoter carrying retroviral vector is suitable for the inducible expression of therapeutic genes and could be employed for gene therapy in the context of cancer chemotherapy.

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Cytosine Deaminase – A suicide system for tumor therapy

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Cytosine Deaminase (CD) is a bacterial and fungal enzyme which is not expressed in mammalian cells. This protein dominates the non-toxic prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU), which is used for chemotherapy of colorectal cancer. Therefore, CD can be used as a suicide system for transfecting tumor cells and subsequent selective killing of CD-transfectants by 5-FC. In order to allow the detection of CD expression at the protein level we generated antibodies against this enzyme. Furthermore, we used a syngeneic rat pancreatic tumor model to examine the CD/5-FC system in *vivo* in immunocompetent animals.

The generated antibodies specifically recognize the CD protein from *E. coli* in various test systems including western blots and immunohistochemistry on frozen tissue sections of rat AS/CD-tumors. For *in vivo* experiments we induced tumors in rats by injection of CD-expressing AS-tumor cells (AS/CD). In comparison to control tumors (AS/neo), which grew rapidly, CD-expressing tumors regressed after initial tumor growth, when exposed to 5-FC or PBS. After a second injection of AS/CD cells and the AS parental cells into these animals AS/CD cells were rejected immediately. Complete regression of the parental tumor was observed in a significant number of animals. A third injection of AS parental cells into the surviving rats showed an immediate rejection of the syngeneic AS tumor cells.

The Abs generated against CD from *E. coli* facilitate the fast and direct detection of the CD protein in transfected cells and tumors. Our preliminary data obtained in the rat tumor model suggest the induction of an immune response by CD – a bacterial antigen – which might modulate the tumor microenvironment such that the originally non-immunogenic parental tumor cells become immunogenic. The molecular basis of this phenomenon is currently under investigation.

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Generation and characterization of cytotoxic T lymphocytes (CTL) against mutated *ras* peptides

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Mutated *ras* genes have been implicated in the initiation and progression of cancers. Therefore, peptides encompassing *ras* mutations appear to represent an appealing target for active immunotherapy procedures. In this work peptides encompassing GLY→VAL, 61 GLN→LEU and 61 GLN→LYS *ras* mutations and displaying HLA-A2.1 binding motifs, selected by a computer program, were used to attempt the generation of specific CTL *in vitro*. Initially, peripheral blood mononuclear cells (PBMC) from five HLA-A2.1+ healthy donors were stimulated *in vitro* with a mixture of peptides. Weekly thereafter, PBMC were restimulated with irradiated peptide pulsed, autologous Epstein Barr virus (EBV) transformed B cells. After eight rounds of restimulation reproducible cytotoxic activity against peptide pulsed target cells was detectable in one donor. CTL recognized two nonamers encompassing *ras* 61 Gln→Leu mutation. Killing was mediated by $CD8^+$ T cells displaying $\alpha\beta$ T cell receptor (TCR) and was inhibited by anti-HLA-A2.1 monoclonal antibodies. None was able to exert effective cytotoxic activity aginst tumor cells expressing the specific mutation.

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Improving tumour targeting and decreasing normal tissue uptake by optimizing stoichiometry of a two-step biotinylated monoclonal antibody (Mab)/streptavidin (Strv) based targeting strategy: Studies in a nude mouse xenograft model

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Purpose: To assess the impact of relative protein stoichiometry of first and second step and biotinylation density of first step on the pharmacokinetics, biodistribution and tumour targeting of a two-step biot-MAb/Strv approach.

Methods: The HT-29 xenograft nude-mouse model was used. AUA1 Mab was biotinylated to various degrees (r. 0.8–25 biotins per IgG). Protein stoichiometry of the two steps was studied through a range of 2 logs. Both steps i.e. the biot-Mab (1st step) and Strv (2nd step) were radiolabelled (^{131}I & ^{125}I). A 24 h interval between 1st/2nd step was studied, animals were killed 24 h after the 2nd step.

Results: Strv excess led to a decrease in circulating levels of biot-Mab (7.6 ± 1.0 vs 11.2 ± 1.3% i.d./g) and decreased amounts of biot-Mab in the tumour (3.7 ± 0.7 vs 5.7 ± 0.6% i.d./g). Biot-Mab excess led to increase in circulating levels of Strv (3.6 ± 0.5 vs 7.5 ± 1.7% i.d./g), a decrease in renal uptake of Strv (66 ± 12 vs 24 ± 5.9% i.d./g) and increased targeting of Strv to tumour (6.9 ± 1.4 vs 5.3 ± 0.9% i.d./g). At a constant protein molar ratio of 1st to 2nd step (10:1 Mab excess), varying IgG biotin density resulted in: (i) The circulating levels of Strv increasing from 4.8 ± 0.6 to 21 ± 2.6% i.d./g (ii) Increase in tumour uptake of biot-MAb (6.2 ± 0.8 vs 24.1 ± 7.7% i.d./g) and (iii) Renal uptake falling from 69 ± 5 to 8.8 ± 3.8% i.d./g while liver uptake increased from 8 ± 1 to 40 ± 14% i.d/g.

Conclusion: Factors pertaining to protein stoichiometry and to biotinylation density of the MAb profoundly affect biodistribution, pharmacokinetics and tumour targetting in two-step based strategies.

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How to perform effective IL-2 therapy

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Locoregional cancer treatment with IL-2 induces good therapeutic effects