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BISPECIFIC ANTIBODY MODIFIED TUMOR VEACCINE FOR HUMAN T CELL STIMULATION

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A new type of cancer vaccine for the therapeutic application in cancer patients is described. It consists of three components. (1) autologous tumor cells, (2) Newcastle Sisease Virus (NDV), to be used for infection and (3) bispecific antibodies (bsAb) which attach to the viral hemagglutinin neuraminidase (HN) molecule on the infected tumor cells A standardized procedure has been developed for generating virus infected human autologous tumor cell vaccines (ATV-NDV) which includes cell dissocation, removal of leokcytes and cell debris, gamma-irradiation and cryopreservation. Infection with the nonvirulent strain NDV Ulster is performed within 30 min of co-incubation. While virus infection already increased immunogenicity of the tumor vaccine, further augmentation of T cell stimulatory capacity is achieved by attachment of specially designed bi-specific antibodies. (bs HN x CD28 or bs HN x CD3). T cell stimulation was achieved in allogeneic and autologous mixed lymphocyte tumor bs-vaccine cultures using responder lymphocytes from peripheral blood of either normal healthy donors or from cancer patients. This tumor vaccine modification with bsAb is highly specific, quick and economic and has broad-range applications.

References:
V. Schirrmacher and C. Haas. Immunogenicity increase of autologous tumor cell vaccines by virus infection and attachment of bispecific antibodies. **Cancer Immunol. Immunother. 43: 190-194 (1996)**

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DENDRITIC CELL-BASED THERAPY FOR CANCER

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Given the wealth of recently identified tumor-associated antigens (TAA) and their corresponding peptide epitopes recognized by MHC-restricted CD8+ T lymphocytes, we are now in a position to carefully evaluate the clinical efficacy of specific TAA-based vaccines for the treatment of various human malignancies. We have previously documented the ability of murine bone marrow-derived dendritic cells (DC) to serve as a "biologic adjuvant" capable of promoting prophylactic and therapeutic anti-tumor immunity when pulsed with relevant synthetic or acid-eluted tumor-associated T-cell epitopes. Furthermore, we have been able to stimulate T-cell responses *in vitro* using human PBMC-derived DC pulsed with melanoma peptide epitopes from MART-1, gp100, or tyrosinase and have designed a clinical protocol in which HLA-A2+ patients with melanoma will receive such modified DCs. Current studies are underway to evaluate the effects of Flt3L and IL12 on DC function *in vitro* and *in vivo* in order to further improve DC-peptide therapies. More recently, we have investigated the possibility to insert genes encoding tumor antigens into DC as a source of tumor antigen. Using a particle-mediated gene transfer technology murine DC were genetically modified to express the tumor antigens HPV16-E_7 or wild-type p53. When applied as a vaccine these genetically modified DC were able to mediate protective anti-tumor immunity *in vivo* upon challenge with relevant tumor cell lines. Human PBMC-derived DC similarly engineered to express the melanoma antigens MART-1, gp100, tyrosinase, MAGE-1, and MAGE-3 were capable of promoting antigen-specific T cell reactivity *in vitro*. Preliminary evidence suggests that antigen-specific CD4+ and CD8+ T cells may be stimulated in this way. Furthermore, the nature of the immune response can be modulated by cotransfecting cytokine genes such as IFN-α or IL-12, suggesting that cytokines present in the DC-T cell priming microenvironment can direct the quality and nature of the resultant anti-tumor immune response. These results provide a basis for the development of cancer vaccines consisting of genetically modified DC for the induction of antitumor immunity.

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DENDRITIC CELL-BASED IMMUNOTHERAPY OF CANCER FROM PRECLINICAL STUDIES TO CLINICAL APPLICATIONS

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Dendritic cells represent potent immunostimulatory antigen presenting cells, capable of optimally priming cytotoxic T lymphocytes *in vitro* and *in vivo*. We have previously shown that bone marrow derived -DC, when cultured in IL-4 and GM-CSF and pulsed *ex vivo* with CTL defined synthetic tumor peptides, serve as effective antitumor vaccines, protecting animals against lethal tumor challenge (Mayordomo *et al.* 1995, Nature Medecine 1: 1297-1302) When no T cell-defined tumor epitope is identified, unfractionated acid-eluted tumor peptides loaded onto DC as cellular adjuvants can be used to treat mice against poorly immunogenic tumors. The adoptive transfer of $5x10^5$ tumor peptide pulsed DC dramatically suppressed day 4 to day 8 established sarcoma and murine breast tumors (MCA205 (H-2b), TS/A (H-2d). In the HPV16-induced C3 tumor model, two iv injections of $5x10^5$ DC could eradicate palpable tumors of 40-50 mm^2. The 205-DC mediated antitumor immune response was CD4 and CD8+T cell-dependent. Similarly, IL-12 and IFNγ+TNFα were critical for the DC-induced antitumor effects as well as the B7/CD28 interactions (Zitvogel *et al.* J.Exp.Med. 183: 87-97, 1996) Based on these mouse data, preclinical studies aimed at investigating the feasability of generating monocyte-derived human DC from circulating mononuclear cells in cancer patients were started. Preliminary experiments show that functional human DC from melanoma and renal cell cancer patients undergoing immunotherapy (IL-12, IFNα+IL-2, GM-CSF) can be derived *ex vivo* and will be discussed.