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Sociology, Population



Immunotherapy of Malignant Melanoma Using Dendritic Cells Vaccines

Abstract

5 research papers on dendritic cell (DC) vaccines for malignant melanoma (MM) were studied. Study designs, patient-specific vaccine preparation, pulsing of DCs and survival rates from different types of DC vaccines were analyzed. Patients with stages III and IV MM were included for trials in all the researches. DCs were pulsed with TRIMEL, tumor lysate, melanoma associated antigens: gp100, MART-1 and tyrosinase. Delayed type hypersensitivity (DTH) was studied to determine effect of the antigen. A positive reaction was considered to be appearance of erythema at the injection site. Pulsed DCs were selected by cloning and selection. ELISPOT was carried out to determine IFN- γ secretion. Routes of vaccination and their effect on efficacy were also explored. Mean survival time differed with usage of each vaccine and there were positive outcomes with certain researchers claiming 50% 5 year mean survival time.

Introduction

Dendritic cells (DCs) originate from the bone marrow and play an important role as antigen presenting cells. DCs have been studied extensively in the field of oncology as they have the ability to elicit differentiation of antigen-specific T cells even in presence of low concentrations of antigen, which is far efficient than macrophages (Steinman, 5). DC presents protein antigen as protein-Major histocompatibility complex (MHC) molecules and lipid antigen as non-classical MHC molecules. DCs present in lymph nodes present the processed antigen to CD4+ cells, which results in production of interleukin-2

(IL-2). This process initiates antigen-specific T cell production. The DC vaccine studies aim at producing these antigen-specific T cells against a specific cancer antigen, thereby eliciting immunogenic response for annihilation of existing cancer cells within the patient's body (Banchereau and Palucka, 266).

Malignant melanoma (MM) has low survival rate when detected at later stages. Use of chemotherapy in combination with drugs has been noted to be toxic and does not change the outcome to a great extent. For this reason, an alternate approach using DC vaccine is being explored. MM produces melanoma associated antigens (MAA or MAGE). Of the various antigens, melanoma associated antigen recognized by T cells (MART-1), gp100 (glycoprotein 100, involved in melanosome maturation), MAGE-3 and tyrosinase are some of the prime candidates for DC vaccine against MM (Lopez, 945). According to Dillman et al, autologous (patient's own cells) DC pulsed with patient-specific antigens from autologous proliferating tumor cells resulted in a mean survival rate of 5 years in 54% of the patients (407). DC vaccine immunotherapy is still at its nascent stages due to certain escape mechanisms implemented by the melanoma cells. One of them is evading the immune system using HLA-A2 negative chain; second is immunosuppression of DCs by the patient's own Treg (regulator T) cells. The current DC vaccine works better for patients who are positive for human leukocyte antigen- A2 (HLA-A2+) rather than a HLA-A2- because, HLA-A2 is responsible for presenting many of the prime candidate MAGE mentioned above to the T cells. Loss of HLA-A2 acts as an escape route for the melanoma cells. Research papers suggest development of slightly different

protocol for HLA-A2- patients (Oshita et al, 1131). Down regulating the Treg cells could bring about a breakthrough in effective delivery of DC vaccines (Svane et al, 1). After more than 10 years of experimenting on MM patients, DC vaccines have been found to be less invasive and safe. To improve the efficacy, various routes of vaccination were also investigated (Lesterhuis et al, 5725).

Methods and materials

In the research conducted by Lopez et al, DCs were pulsed with a new allogenic tumor cell lysate called TRIMEL. 12 patients were vaccinated on 0th, 10th, 30th and 50th day from the start of the trial. DTH reactions testing were checked using sub-cutaneous injection of the antigens. Immune-monitoring was done using ELISPOT. To analyze T-cell population before and after vaccination protocol, peripheral blood mononuclear cells (PBMCs) from the patients were obtained through leukapheresis (removal of selective white blood cells from the peripheral blood) and treated with appropriate monoclonal antibodies (MAbs). The resultant was studied using flowcytometry (945-46). According to research done by Oshita et al, 24 patients were vaccinated with DC-pulsed with a mixture of 5 melanoma specific antigens; MART-1, gp100, tyrosinase, MAGE-A2, and MAGE-A3 for HLA-A2+ and gp100, tyrosinase, MAGE-A1, MAGE-A2 and MAGE-A3 for HLA-A2-. Delayed type hypersensitivity (DTH) was checked as described above. Monitoring of the vaccinated patients was done using ELISPOT assay and ELISA (1132).

Svane et al obtained patient specific melanoma antigens from peripheral blood of patients, collected before treatment, on 4th, 6th, 10th day and

every 3 months (2). HLA-A2+ patients were vaccinated with DC-pulsed with p53, survivin and telomerase derived peptides. HLA-A2- patients received DC pulsed with tumor lysate. First 4 vaccines were given once a week. Next 6 vaccines were given once on alternate weeks. Apart from the primary therapy, a secondary adjuvant treatment was given using cyclophosphamide (Cy), Celecoxib and IL-2. Since the study's purpose was to analyze the influence of Treg population in the 3 trials, flowcytometry analysis were done using patients sample after receiving of the DC vaccine and adjuvant treatments (Svane et al, 2).

Lesterhuis et al studied whether route of administration of the DC vaccines have any influence on the efficacy of the treatment. HLA-A2+ patients expressing the MAA gp100 and tyrosinase, scheduled for regional lymph node dissection were selected. They were given intranodal (IN) and intradermal (ID) DC vaccines pulsed with gp100, tyrosinase and keyhole limpet hemocyanin (KLH). For measuring DC migration using scintigraphic imaging, a prior dose of radio-nucleotide was injected. The patients were divided into 4 groups: ID without IL-2 treatment, ID with IL-2 treatment, IN without IL-2 treatment and IN with IL-2 treatment (5727-28).

The research by Dillman et al points out the factors as to why DC vaccines might be effective against melanoma. The experimental design consisted of characterizing the PBMCs obtained through leukapheresis, quantity of DCs administered, changes in the DCs in a culture, characters of the tumor cells used (414).

Tumor lysate prep

Tumor lysate is prepared from a continuous melanoma cell line by cloning and selection and selecting cells that express MAGE antigens but not tyrosinase, gp100 and MART-1. The cell lines are tested for contamination (presence of Hepatitis B & C, HIV 1 & 2 and mycoplasma). Selected cells are grown in RPMI 1640 medium with 2% human AB serum followed by growth in serum-free PC-1 medium. For harvesting, commercial cell dissociation reagent such as Versene is used. The cells are washed off the residual chemical using RPMI 1640 and suspended in the same at a cell concentration of 10⁷ cells/ml. 5 freezing/thawing cycles are implemented on the final cell product. The tumor cell lysate is centrifuged at 400g for 10 min followed by 10000g for 30 min. The supernatant is then filtered and stored in aliquots at -80°C until further use (Burgdorf et al, 202).

Pulsing of DCs and vaccine preparation

Patient-specific DC vaccines were created from tumor cells derived from continuously proliferating tumor cell lines. The cells were increased in number by treating the cells for 72 hours with 1000U/mL interferon-gamma (IFN- γ) and DCs. DCs were isolated from PBMCs of the patient. Upon co-culturing of DCs with the tumor cells in granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL 4, the DCs phagocytosed the tumor cells, thereby producing the resultant patient-specific DC vaccine. These DC vaccines were stored in individual aliquots in liquid nitrogen until further use (Dillman et al, 409).

Results and important findings

The research by Lopez et al using TRIMEL pulsed DC vaccine on 43 stage IV MM patients shows a mean survival rate of 15 months. No tumor relapse was seen in all the 7 stage III MM patients during the 2 year follow up period (948). The mean survival time using multiple antigen (MART-1, gp100, tyrosinase, MAGE-A2, and MAGE-A3 for HLA-A2+ and gp100, tyrosinase, MAGE-A1, MAGE-A2 and MAGE-A3 for HLA-A2-) was 13.6 months (Oshita et al, 1134). The mean survival rate using tumor cell lysate-pulsed DCs and multiple variants was 1 year in 85% of the patients and 5 years for 50% of the patients (Dillman et al, 411). Treatment with metronomic cyclophosphamide along with IL-2 and DCs was carried out in hopes of decreasing the Treg population, which otherwise hinders with the immunotherapy using DC vaccine. The Treg population increased upon treatment with IL-2. The numbers also increased upon treatment with only MCy and DC-MCy-IL-2 therapy and reduced only a little by the 6th vaccine (Svane et al, 6).

Route of vaccination had some role to play as per the research by Lesterhuis et al. ID vaccination without IL-2 resulted in mean survival of 32 months (2-61 months); ID vaccinations with IL-2 resulted in higher Treg population and mean survival of 27 months (6-115 months); IN vaccination without IL-2 gave mean survival of 42 months (7-74 months) and IN vaccination with IL-2 resulted in mean survival of 14 months (4-83 months) (5729).

Discussion

TRIMEL pulsed DC vaccine was found effective against stage III and IV MM patients. Multiple antigens pulsed DCs had moderate effect on the tumor in

which among 24 patients, 16 had progressive disease, 7 had stable disease (no growth, yet no reduction) and 1 had partial remission of the tumor. Tumor lysate pulse DC vaccine has shown significantly higher mean survival rate. Intradermal vaccination was found to be more effective than intranodal vaccination. Concomitant treatment with IL-2 did not augment either of the vaccination process. IL-2 also seemed to increase the Treg population, thereby hindering immunotherapy. Efficacy of the vaccine was directly proportional to the number of viable cells in the vaccine. The presence of non-viable tumor cell could also be a contributing factor the final vaccine product.

Conclusion

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