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(54) Title: CANCER VACCINE TREATMENT BY SYNCHRONOUSLY CULTURING AND IMMUNIZING OF AUTOLOGOUS NK (NATURAL KILLER) CELLS WITH AUTOLOGOUS CANCER CELLS

(57) Abstract: Glioblastoma multiforme (GBM) and anaplastic astrocytoma are the most malignant and most common astrocytic brain tumors, with an incidence of 5 / 100,000 and pro-neuronal, neural, classic, mesenchymal types. There is no definitive treatment and the average life expectancy is 45-71 weeks. The recurrence period is around 9-36 weeks, and radical surgical resection, radio-chemotherapy (Temozolamide, 6-12 cycles) is the standard of care, causing minimal functional and structural loss; In case of recurrence, re-surgery and bevacizumab treatment are performed in appropriate cases. The proposed vaccine aims to prevent recurrence and definitive treatment in the treatment of GBM and anaplastic astrocytoma and in the treatment of some Grade 2 astrocytomas. However, this approach can be applied to all human solid cancers.

CANCER VACCINE TREATMENT BY SYNCHRONOUSLY CULTURING AND IMMUNIZING OF AUTOLOGOUS NK (NATURAL KILLER) CELLS WITH AUTOLOGOUS CANCER CELLS

TECHNICAL FIELD

The invention relates to the use of cancer vaccine treatment by synchronously culturing and immunizing of autologous nk *(natural killer)* cells with autologous cancer cells with the aim of preventing recurrence and definitive treatment in GBM and anaplastic astrocytoma and in the treatment of certain Grade 2 astrocytomas.

STATE OF ART

Radio-chemotherapy, molecular therapies (pilumimab, nivolumab, and pembrolizumab, etc.), gene and vaccine therapies after surgical treatment are not able to cure **Glioblastoma multiforme** (GBM) precisely and 95% of the patients die within the first 2 years after the first diagnosis. Existing treatments are not specific enough ; biological complexity, a big amount of impaired intracellular signaling pathways, and the lack of a universal molecular marker particular to this type of cancer, in other words, the absence of the therapeutic target is the major shortcomings at the moment. Interestingly, although there is no lymphatic system in brain tissue, there is lymphocyte infiltration around GBM cells. Based on this, it is understood that GBM causes immunosuppression and the tumor masks itself (tumor escape).

1- Decreases MHC-1 levels,

2- Increases the amount of immune control point regulatory factors (PD-1, TGF-B, VEGF, IL-10 etc.),

3- The immune suppressor cells Treg and M2 and tumor-dependent macrophages are drawn into the medium suppresses effector T cells.

There is presently no efficient vaccine in the therapy of GBM that totally eliminates the barriers described above.

Said invention, in EP 2 341 927 B1 relates to peptides, nucleic acids and cells used in immunotherapeutic methods. In particular, the invention relates to cancer

immunotherapy. The invention also relates to tumor-associated cytotoxic T cell (CTC) peptide epitopes that are used as active pharmaceutical ingredients of vaccine compositions that promote anti-tumor immune responses in combination with or alone with other tumor-associated peptides. The existing invention relates to 30 peptide sequences and their variants derived from human tumor cell molecules of HLA Class I and Class II that can be used in vaccine compositions to generate anti-tumor immune responses. It is a peptide-based vaccine for the treatment of GBM and other cancers and a tumor-associated peptide composition. The invention described above belongs to the peptide-based cancer vaccine group in the prior art.

DESCRIPTION OF THE INVENTION

The present invention relates to the cancer vaccine treatment by synchronously culturing and immunizing of autologous nk (natural killer) cells with autologous cancer cells developed to eliminate the above-mentioned disadvantages and bring new advantages to the relevant field.

The vaccine of the invention is based on the principle of specifically "immunizing" the patient's Natural Killer (NK) cells in co-cell culture as a whole with GBM cells and reintroducing them to the patient.

Gliomir: Cancer tissue samples of human brain cancers (GBM, anaplastic astrocytoma, medulloblastoma, pineoblastoma etc. and some grade 2 astrocytomas) and other human organ cancers (lung, prostate, melanoma, breast, pancreas, stomach, colon, others) obtained by surgery and the cell culture of the Natural Killer lymphocyte group (NK) synchronously isolated from the peripheral venous blood (NK) will be co-cultured with the cancer cells for 2 weeks to 2 months and thus the NK cells cells will be immunized against the tumor cells. NK cells will be isolated from tumor cells using appropriate assays and, if ethical committee approval is obtained, patients may be given subcutaneously, subcutaneously, intravenously / intraarterially (intraarticularly) or intracavitaryly. This procedure can be repeated several times if necessary using immunized autologous NKs stored and cell passages, and the healing of patients will be monitored clinically and radiologically.

In this detailed description, preferred alternatives to cancer vaccine treatment by

synchronously culturisation and immunization of autologous nk (natural killer) cells with autologous cancer cells of the invention are described only for better understanding of the subject and with no limiting effect.

Experimented brain cancer (gliom) vaccines

Gliovac; Radiation-inactivated autologous-GBM cells and three different allogeneic GBM cell groups are combined with GM-CSF and priorly low-dose cyclophosphamide treatment is given. Schijns et al. tested the vaccine in 9 relapsed GBM patients; In this group, 40-week survival is (77%) - in the group without GlioVac is (10%). (Vaccine; 2015 May 28; 33 (23): 2690-6. doi: 10.1016 / j.vaccine.2015.03.095. First clinical results of a personalized immunotherapeutic vaccine against recurrent, incompletely resected, treatment-resistant glioblastoma multiforme (GBM) tumors, based on combined alio- and auto-immune tumor reactivity). 6 months after relapse; all patients in the vaccine group and only one third of the patients in the control group survived. There was no definite treatment.

EGFRvIII vaccine practice generated against human cytomegalovirus (CMV) -derived antigens, IDH-1 (R132H), IL13Ra2, HER-2, gp100, TRP2, EphA2, survivin, WT1, SOX2, SOX11, MAGE-A1.MAGE-A3, AIM2, SART1 antigens: In the study of 12 recurrent GBM patients, autologous dendritic cells were introduced to the vaccine site in order to strengthen the immune response by pre-administering known tetanus / diphtheria toxoid-like immunomodulatory antigens and then introducing CMV phosphoprotein 65 (pp65) introduced in vitro; There was a significant increase in time to recurrence and survival. (Mitchell DA, et al. Tetanus toxoid and CCL3improve dendritic cell vaccines in mice and glioblastoma patients. Nature.2015;519D7543]:366-9. doi:10.1038/nature14320).

The mutation of isocitrate dehydrogenase-1 (IDH1) (R132H) is another tumor-specific antigen vaccine (Phase 1) that is currently remain under investigation. (Schumacher *T,* et al. A vaccine targeting mutant IDH1 induces antitumour immunity. Nature.2014;512D7514]:324-7. doi: 10.1038/nature13387.)

Peptide vaccines-Rindopepimut (Celldex Therapeutics): It is the most advanced peptide vaccine which consists of synthetically obtained, mutated epidermal growth

factor receptor (EGFRIII) antigen with the combination of "Keyhole limpet hemocyanin" as carrier protein and co-administration with GM-SCF and which Phase 1, 2 and 3 trials still in progress. EGFRvII1 is present in approximately 30% of GBM patients and forms a target for the vaccine by forming a neo-antigenic junction not found in normal cells after deletion.

Flumoral and cellular immune responses are triggered, and in the ongoing Phase 2 trial, rindopepimut is combined with bevacizumab. Recently, in a controlled study, the rindopepimut group showed a 2-month difference in survival after relapse compared to the placebo group.

Patient-specific peptide vaccines: As a result of "DNA sequencing", mutations and neo-antigens of that tumor tissue are detected and more efficient peptide-based vaccine development studies are being tested in melanomas; there is a similar trials in progress for GBM. (Enova, Clinicatrials.gov:NCT01903330).

Explanations Not Included in the Patent Literature:

Oncoimmunolgy 2015, 21;5(4):e1119354. Dual targeting of glioblastoma with chimeric antigen receptor-engineered natural killer cells overcomes heterogeneity of target antigen expression and enhances antitumor activity and survival. Gensler S, et al.

J Natl Cancer Inst. 2016,6;108(5). ErbB2/HER2-Specific NK Cells for Targeted Therapy of Glioblastoma. Zhang C et al.

Sanchez-Perez LA, et al. Myeloablative temozolomide enhances CD8(+) T-cell responses to vaccine and is required for efficacy against brain tumors in mice. PLoS One. 2013;8(3), e59082.

CLAIMS

1. **The subject of the invention is a method of cancer vaccine and treatment method** obtained by synchronously culturing and immunizing of autologous nk (natural killer) cells with autologous cancer cells, **characterized by**, specifically "immunizing" the patient's Natural Killer (NK) cells in co-cell culture as a whole with GBM cells and reintroducing them to the patient.

2. The invention is a cancer vaccine and treatment method according to Claim 1, **characterized** by following steps:
   - Taking cancer tissue samples obtained by surgery,
   - Synchronously isolation of samples from peripheral venous blood,
   - Cell culture of the Natural Killer lymphocyte group (NK) synchronously with cancer cells (co-culture) between 2 weeks and 2 months,
   - Ensuring that NK cells are immunized against tumor cells,
   - Isolating NK cells from tumor cells using appropriate assays,
   - Subcutaneous, intra-venous / intra-arterial, intracavitary (intratumoral) vaccination to patients,
   - Repeating this procedure recurrently using immunized autologous NKs stored and cell passages made,
   - The clinical and radiological follow-up of patients' healing processes.

3. The invention is a cancer vaccine and treatment method according to Claim 1 and Claim 2, **characterized in that** it can be applied with multiple repetitions in certain periods by using immunized autologous Natural Killer cells.

4. The invention is a cancer vaccine and treatment method according to any one of the preceding claims, **characterized** by the treatment method can be applied to GBM, anaplastic astrocytoma, medulloblastoma, pineoblastoma etc. and some grade 2 astrocytomas and also other human organ cancers such as lung, prostate, melanoma, breast, pancreas, stomach, colon, and other cancers.

5. The invention is a cancer vaccine and treatment method according to any one of

the preceding claims, **characterized** by the obtained vaccine can be applied to GBM, anaplastic astrocytoma, medulloblastoma, pineoblastoma etc. and some grade 2 astrocytomas and also other human organ cancers such as lung, prostate, melanoma, breast, pancreas, stomach, colon, and other cancers.

6. The invention is a cancer vaccine and treatment method according to any one of the preceding claims, **characterized** by the vaccine can be produced and / or prepared specifically for each patient.

7. The invention is a cancer vaccine and treatment method according to any one of the preceding claims, **characterized** by the vaccine can be vaccinate to patients subcutaneously (intra-dermally), intravenously / intra-arterially and intracavitary (intratumorally).