Description

Title of Invention: METHOD FOR TREATING CERVICAL CANCER

Technical Field

[1] This disclosure relates to a pharmaceutical composition comprising (a) a human papillomavirus (HPV) vaccine; and (b) a checkpoint inhibitor for preventing or treating a human papillomavirus (HPV)-induced cancer.the treatment of cervical cancer caused by human papillomavirus (HPV) infection.

[2]

Background Art

[3] Persistent viral infection often induces functional inactivation of virus-specific CD8 T cells, impairing their capacity to proliferate, produce immune-stimulatory cytokines, and lyse virally infected cells. Cervical cancer is one of the leading causes of cancer death in women worldwide, and about 75% of its cases are caused by persistent infection with the most common high-risk human papillomavirus (HPV) types, namely HPV16 and HPV18. HPV persistence is usually associated with the lack of demonstrable HPV-specific T-cell immunity, and the virus-specific T cells found in pre-malignant and malignant patients are reported to be generally dysfunctional and sometimes even suppressive. These findings suggest that the functional impairment of virus-specific T cells might be associated with the emergence of HPV-induced cervical cancer.

[4] Cervical cancer arises via a course of high-risk HPV infection, viral persistence, clonal expansion and differentiation of persistently infected cells to a pre-malignant lesion, and their gradual transformation into invasive cancer.

[5] According to World Health Organization (WHO), cervical cancer is the fourth most frequent cancer in women with an estimated 570,000 new cases in 2018 representing 7.5% of all female cancer deaths. The pre-malignant cervical intraepithelial neoplasia 2 and 3 (CIN2 and 3), in particular those positive for HPV16, are considered as high-grade lesions that have approximately a 30% chance of developing into invasive cancer. Therefore, there is urgent need for an effective therapeutic vaccine that can prevent severe complication of persistent HPV infection and eradicate HPV-related neoplasia.

[6] HPV E6 and E7 proteins act as viral oncoproteins by binding and promoting degradation of tumor suppressor proteins, p53 and retinoblastoma (pRb), respectively. These viral oncoproteins are an ideal set of targets for a therapeutic vaccine against CIN2/3 and cervical cancer not only because these proteins induce tumorigenesis but

they are also constitutively expressed in HPV-infected pre-malignant and malignant cells. Since the regression of cervical lesions is associated with the presence of a cellular, but not humoral, immune response, a therapeutic vaccine capable of selectively inducing robust E6/E7-specific T-cell immunity is highly desirable.

[7] As one of the ongoing efforts to provide an effective prevention/treatment of cervical cancer caused by HPV, a fusion protein comprising three or more amino acid sequences selected from: (1) an N-terminal portion of an E6 protein of HPV16, (2) a C-terminal portion of an E6 protein of HPV16, (3) an N-terminal portion of an E7 protein of HPV16, (4) a C-terminal portion of an E7 protein of HPV16, (5) an N-terminal portion of an E6 protein of HPV18, (6) a C-terminal portion of an E6 protein of HPV18, (7) an N-terminal portion of an E7 protein of HPV18, and (8) a C-terminal portion of an E7 protein of HPV18, wherein the fusion protein does not bind to p53 or does not form a dimer with an E6 protein of HPV16 or HPV18 and wherein the fusion protein does not bind to pRb or does not form a dimer with an E7 protein of HPV16 or HPV18 was described in co-pending application no. 15/503,997. The entire content of co-pending application no. 15/503,997 is incorporated herein by reference.

[8] A fusion protein including a fusion polypeptide configured to transform a 3D structure of E6 and E7 derived from HPV types 16 and 18, and an immunity enhancer peptide, and a polynucleotide encoding the fusion protein are disclosed in co-pending US application no. 13/816,716. A fusion protein according to one exemplary embodiment of co-pending US application no. 13/816,716 may include a fusion polypeptide recombined to transform a 3D structure of the E6 and E7 derived from the HPV types 16 and 18. More particularly, the fusion polypeptide is a fusion polypeptide in which 1st to 85th amino acids of the E6 protein derived from the HPV type 16, 1st to 65th amino acids of the E7 protein, 71st to 158th amino acids of the E6 protein, and 51st to 98th amino acids of the E7 protein, 1st to 85th amino acids of the E6 protein derived from the HPV type 18, 1st to 65th amino acids of the E7 protein, 71st to 158th amino acids of the E6 protein, and 51st to 105th amino acids of the E7 protein are bound in sequence. In an embodiment of co-pending US application no. 13/816,716 is directed to a nucleic acid molecule encoding the fusion polypeptide and an immunity enhancer peptide. Exemplary immunity enhancer peptide includes CD40 ligand, Flt3 ligand, flagellin, and/or OX40. An embodiment of a polynucleotide encoding the fusion protein, an optimized signal sequence (e.g., tPa), and an immunity enhancer peptide (e.g., Flt3 ligand) may be manufactured by reference to Example 1 of co-pending application no. 13/816,716. The entire content of co-pending US application no. 13/816,716 is incorporated herein by reference.

[9] Immunotherapy, which enhances the body's own immune system to enable the body to amplify an immune response against cancer cells, can boost or change how the

immune system works so it can find and attack cancer cells.

[10] Studies with checkpoint inhibitor antibodies for cancer therapy have generated unprecedented response rates in cancers previously thought to be resistant to cancer treatment. Therapy with antagonistic checkpoint blocking antibodies against CTLA-4, PD-1 and PD-L1 are one of the most promising new avenues of immunotherapy for cancer and other diseases. In contrast to the majority of anti-cancer agents, checkpoint inhibitors do not target tumor cells directly, but rather target lymphocyte receptors or their ligands in order to enhance the endogenous antitumor activity of the immune system. Because immune checkpoint antibodies act primarily by regulating the immune response to diseased cells, tissues or pathogens, they may be used in combination with other therapeutic modalities, such as antibody-drug conjugates to enhance the anti-tumor effect of the antibody-drug conjugates.

[11] Programmed cell death protein 1 (PD-1, also known as CD279) encodes a cell surface membrane protein of the immunoglobulin superfamily, which is expressed in B cells and NK cells. Anti-PD1 antibodies have been used for treatment of melanoma, non-small-cell lung cancer, bladder cancer, prostate cancer, colorectal cancer, head and neck cancer, triple-negative breast cancer, leukemia, lymphoma and renal cell cancer. Exemplary anti-PD1 antibodies include pembrolizumab (KEYTRUDA®, MERCK), nivolumab (BMS-936558, BRISTOL-MYERS SQUIBB), cemiplimab (LIBTAYO®), and pidilizumab (CT-011, CURETECH LTD.).

[12] Programmed cell death 1 ligand 1 (PD-L1, also known as CD274) is a ligand for PD-1, found on activated T cells, B cells, myeloid cells and macrophages. The complex of PD-1 and PD-L1 inhibits proliferation of CD8+ T cells and reduces the immune response. Anti-PD-L1 antibodies have been used for treatment of non-small cell lung cancer, melanoma, colorectal cancer, renal-cell cancer, pancreatic cancer, gastric cancer, ovarian cancer, breast cancer, and hematologic malignancies. Exemplary anti-PD-L1 antibodies include MDX-1105 (MEDAREX), durvalumab (MEDI4736, MEDIMMUNE), avelumab (BAVENCIO®), atezolizumab (TECENTRIQ®, MPDL3280A, GENENTECH) and BMS-936559 (BRISTOL-MYERS SQUIBB).

[13] Cytotoxic T-lymphocyte antigen 4 (CTLA-4, also known as CD152) is also a member of the immunoglobulin superfamily that is expressed exclusively on T-cells. CTLA-4 acts to inhibit T cell activation and is reported to inhibit helper T cell activity and enhance regulatory T cell immunosuppressive activity. Exemplary anti-CTLA4 antibodies include ipilimumab (Bristol-Myers Squibb) and tremelimumab (PFIZER).

[14] Pembrolizumab (KEYTRUDA®, Merck and Co. Inc.) was recently approved for patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy whose tumors express PD-L1 (CPS $\geq$ 1) as determined by an FDA-approved test. The major efficacy outcomes were objective response rate (ORR)

according to RECIST 1.1 as assessed by blinded independent central review, and response duration. With a median follow-up time of 11.7 months, the ORR in 77 patients was 14.3% (95% CI: 7.4, 24.1), including 2.6% complete responses and 11.7% partial responses. The estimated median response duration based on 11 patients with a response by independent review was not reached (range 4.1, 18.6+ months); 91% had a response duration of greater than or equal to 6 months. No responses were observed in patients whose tumors did not have PD-L1 expression (CPS 1). The most common adverse reactions in at least 10% of patients with cervical cancer enrolled in clinical trial (KEYNOTE-158) were fatigue, pain, pyrexia, peripheral edema, musculoskeletal pain, diarrhea/colitis, abdominal pain, nausea, vomiting, constipation, decreased appetite, hemorrhage, urinary tract infection (UTI), infections, rash, hypothyroidism, headache, and dyspnea. Pembrolizumab was discontinued due to adverse reactions in 8% of patients. Serious adverse reactions occurred in 39% of patients. The most frequent serious adverse reactions reported included anemia (7%), fistula (4.1%), hemorrhage (4.1%), and infections (except UTIs) (4.1%).

[15] However, there is still need for effective treatment and/or enhancing a treatment of cervical cancer, of which about 70% is caused by HPV 16 and/or 18 infection, and/or a cervical cancer in a patient who is PD-L1 negative.

[16]

Disclosure of Invention

Technical Problem

[17] An aspect of the instant disclosure involves the combined administration of an HPV vaccine therapy and an anti-PD1 checkpoint inhibitory antibody therapy over a common time period, after extensive experimentation, was found to increase the suppressive effect on the growth of cervical cancer, in particular advanced, metastatic, recurrent cervical cancer, compared to anti-PD1 checkpoint inhibitory antibody therapy. The instant combination treatment shows an enhancement in treatment efficacy in PD-1 positive patients, HPV-16 positive and/or HPV-17 positive patients than HPV vaccine alone or anti-PD1 checkpoint inhibitory antibody monotherapy. Surprisingly the instant combination therapy further shows a treatment efficacy in PD-1 negative patients, who are not responsive at all in anti-PD1 antibody monotherapy.

[18] An object of the instant disclosure involves a pharmaceutical composition comprising a human papillomavirus (HPV) vaccine and a checkpoint inhibitor agent for preventing or treating a human papillomavirus (HPV)-induced cancer.

[19] Another object of the instant disclosure involves a use of a pharmaceutical composition comprising a human papillomavirus (HPV) vaccine and a checkpoint inhibitor agent in the manufacture of a medicament for preventing or treating a human papil-

lomavirus (HPV)-induced cancer.

[20] Another object of the instant disclosure involves a method for treating human papillomavirus (HPV)-induced cancer in a subject in need thereof, comprising administering an HPV vaccine and a checkpoint inhibitor agent to the subject, over a common period of time, for generating a therapeutic effect greater than either the HPV vaccine or checkpoint inhibitor alone when used as monotherapy.

[21]

Solution to Problem

[22] The instant disclosure is directed to a combination treatment of cervical cancer, comprising administering to a subject with cervical cancer a fusion protein or a DNA vaccine as described herein and an immunomodulator compound, simultaneously or sequentially. In an embodiment, the cervical cancer is caused by HPV infection.

[23] The instant disclosure is directed to a combination treatment of a cancer comprising administering an effective amount of a HPV vaccine and a checkpoint inhibitor agent, in combination, to treat or enhance the treatment of cervical cancer in a subject in need thereof. In an embodiment, the cervical cancer is caused by HPV infection.

[24] The instant disclosure is directed to a use of a HPV vaccine and a checkpoint inhibitor agent, in combination, in a method to treat or enhance the treatment of cervical cancer in a subject in need thereof, wherein the method comprises administering an effective amount of the HPV vaccine and an effective amount of the checkpoint inhibitor agent to an individual in need of enhancing a treatment or treating a cervical cancer. In an embodiment, the cervical cancer is caused by HPV infection. In an embodiment, the individual is PD-L1 positive or negative. In still an embodiment, the individual is PD-L1 negative. In another embodiment, the individual is PD-L1 positive and infected with HPV 16. In an embodiment, the cervical cancer is advanced, recurrent, or metastatic cervical cancer. In an embodiment, cervical cancer is squamous cell carcinoma or adenocarcinoma. In an embodiment, the individual may be PL-L1 positive and infected with HPV 16, and suffers from squamous cell carcinoma.

[25] The instant disclosure is directed to a combinational therapy composition for treating or enhancing treatment of cervical cancer, in which the combinational therapy composition comprises an effective amount of a HPV vaccine and an effective amount of a checkpoint inhibitor agent, wherein the HPV vaccine and the checkpoint inhibitor are administered simultaneously, separately, or sequentially, to an individual in need thereof. In an embodiment, the cervical cancer is caused by HPV infection. In an embodiment, the individual is PD-L1 positive or negative. In still an embodiment, the individual is PD-L1 negative. In another embodiment, the individual is PD-L1 positive and infected with HPV 16. In an embodiment, the cervical cancer is advanced,

recurrent, or metastatic cervical cancer. In an embodiment, cervical cancer is squamous cell carcinoma or adenocarcinoma. In an embodiment, the individual may be PL-L1 positive and infected with HPV 16, and suffers from squamous cell carcinoma.

[26] The instant disclosure is directed to a combinational therapy composition for treating or enhancing treatment of cervical cancer, in which the combinational therapy composition consisting essentially of an effective amount of a HPV vaccine and an effective amount of a checkpoint inhibitor agent, wherein the HPV vaccine and the checkpoint inhibitor are administered simultaneously, separately, or sequentially, to an individual in need thereof. In an embodiment, the cervical cancer is caused by HPV infection. In an embodiment, the individual is PD-L1 positive or negative. In still an embodiment, the individual is PD-L1 negative. In another embodiment, the individual is PD-L1 positive and infected with HPV 16. In an embodiment, the cervical cancer is advanced, recurrent, or metastatic cervical cancer. In an embodiment, cervical cancer is squamous cell carcinoma or adenocarcinoma. In an embodiment, the individual may be PL-L1 positive and infected with HPV 16, and suffers from squamous cell carcinoma.

[27] The instant disclosure is directed to a pharmaceutical composition comprising (a) a human papillomavirus (HPV) vaccine; and (b) a checkpoint inhibitor for preventing or treating a human papillomavirus (HPV)-induced cancer, wherein (a) the HPV vaccine therapy component adapted to be administered to the subject including additional boosts of the HPV vaccine, and (b) the checkpoint inhibitor therapy component adapted to be administered to the subject including additional boosts of the checkpoint inhibitor therapy, wherein at therapeutically effective amounts of each of said components (a) and (b), the combined administrations have the capacity to increase the subject's immune response to treat the HPV-induced cancer, over any increase of the individual's immune response by administration of either of the component (a) or (b) alone. In an embodiment, the subject is PD-L1 positive or negative. In still an embodiment, the individual is PD-L1 negative. In another embodiment, the individual is PD-L1 positive and infected with HPV 16. In an embodiment, the cervical cancer is advanced, recurrent, or metastatic cervical cancer. In an embodiment, cervical cancer is squamous cell carcinoma or adenocarcinoma. In an embodiment, the individual may be PL-L1 positive and infected with HPV 16, and suffers from squamous cell carcinoma.

[28] TThe instant disclosure is directed to a pharmaceutical composistion for treating human papillomavirus (HPV)-induced cancer in a subject in need thereof, consisting essentially of (a) a HPV vaccine therapy component adapted to be administered to the subject including additional boosts of the HPV vaccine, and (b) a checkpoint inhibitor therapy component adapted to be administered to the subject including additional boosts of the checkpoint inhibitor therapy, wherein at therapeutically effective amounts of each of said components (a) and (b), the combined administrations have the capacity

to increase the subject's immune response to treat the HPV-induced cancer, over any increase of the individual's immune response by administration of either of the component (a) or (b) alone. In an embodiment, the subject is PD-L1 positive or negative. In still an embodiment, the individual is PD-L1 negative. In another embodiment, the individual is PD-L1 positive and infected with HPV 16. In an embodiment, the cervical cancer is advanced, recurrent, or metastatic cervical cancer. In an embodiment, cervical cancer is squamous cell carcinoma or adenocarcinoma. In an embodiment, the individual may be PL-L1 positive and infected with HPV 16, and suffers from squamous cell carcinoma.

[29] The instant disclosure is further directed to a pharmaceutical coomposition for treating a human papillomavirus (HPV)-cancer patient comprising two separate therapeutic components, including (a) multiple ones of an HPV vaccine therapy component comprising a polynucleotide of SEQ ID NO: 9, to be administered at a second dose level, and (b) multiple ones of a checkpoint inhibitor therapy component to be administered at one dose level, wherein the HPV vaccine therapy component (a) is to be administered in combination with the checkpoint inhibitor therapy component (b), wherein each of the components (a) and (b) respectively configured at an effective amount at the first and second dose levels, to increase the immune response of the patient by increasing the potency of the checkpoint inhibitor therapy component (b), to provide a benefit of an enhanced immune response over each of the components (a) and (b) administered alone as monotherapy.

[30] The instant disclosure is directed to a use of a pharmaceutical composition comprising (a) a human papillomavirus (HPV) vaccine; and (b) a checkpoint inhibitor agent in the manufacture of a medicament for preventing or treating a human papillomavirus (HPV)-induced cancer.

[31] The present disclosure is directed to a use of a pharmaceutical combination in a method of treating a human papillomavirus (HPV)-induced cancer treatment, said method comprising administering an HPV vaccine and a checkpoint inhibitor agent to a subject in need thereof, over a common period of time, for generating a therapeutic effect greater than either the HPV vaccine or checkpoint inhibitor alone when used as monotherapy.

[32] In an aspect of the above-described methods, combination treatments, the uses, the compositions and the pharmaceutical compositions, the HPV vaccine may be a DNA vaccine. In an embodiment, the checkpoint inhibitor is may be a monoclonal antibody. Exemplary embodiments of the monoclonal antibody may be an anti-PD1 antibody, anti-PD-L1 antibody, or anti-PD1/PD-L1 antibody, or a combination thereof. In an embodiment, the checkpoint inhibitor may be pembrolizumab. In an embodiment, the HPV vaccine is a DNA vaccine comprising the polynucleotide of SEQ ID NO: 15 or a

variant with sequence identity of about 85% or more, about 86% or more, about 87% or more, about 88% or more, about 89% or more, about 90% or more, about 91% or more, about 92% or more, about 93% or more, about 94% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, about 99% or more, or about 100% to SEQ ID NO: 15.

[33] In an aspect of the above-described methods, combination treatments, the uses, the compositions and the pharmaceutical compositions, the subject, individual, or patient may have advanced, inoperable, or metastatic cervical cancer. The cancer may be recurrent. In an embodiment, the cancer is squamous cell carcinoma or adeno-carcinoma. In an embodiment, the cancer is squamous cell carcinoma.

[34] In an aspect of the above-described methods, combination treatments, the uses, the compositions and the pharmaceutical compositions, the subject, individual, or patient is HPV 16 and/or HPV 18-positive. In another embodiment, the individual is PD-L1 positive and infected with HPV 16. In an embodiment, the individual may be PL-L1 positive and infected with HPV 16, and suffers from squamous cell carcinoma.

[35] In an aspect of the above-described methods, combination treatments, the uses, the compositions, and the pharmaceutical compositions, the subject, individual, or patient has been or is treated with anti-cancer treatment. The anti-cancer treatment may be one of known treatments. In an embodiment, the subject, individual, or patient might have undergone or is taking at least one chemotherapy.

[36] In an aspect of the above-described methods, combination treatments, the uses, the compositions, and the pharmaceutical compositions, the subject, individual, or patient may be PD-L1-positive or PD-1L negative. In another embodiment, the subject, individual, or patient may be HPV 16 positive. In still another embodiment, the subject may be PD-L1-positive and HPV 16 positive, and the cervical cancer is squamous cell carcinoma.

[37] In an aspect of the above-described methods, combination treatments, the uses, the compositions, and the pharmaceutical compositions, the HPV vaccine may be GX-188 or GX-188 variant. GX-188E is a deoxyribonucleic acid construct comprising the sequence of SEQ ID NO: 15 that comprises the sequence encoding an E6/E7 fusion protein of HPV 16 and 18 (SEQ ID NO: 9) coupled to tPA and Flt3L (see, FIG. 1). In an embodiment, about 2 mg of GX-188E may be intramuscularly administered at weeks 1, 2, 4, 7, 13, 19, and optional dose at week 46.

[38] In an aspect of the above-described methods, combination treatments, the uses, the compositions, and the pharmaceutical compositions, the checkpoint inhibitor agent may be pembrolizumab and the dose of the checkpoint inhibitor agent may be about 200 mg every three weeks. In an embodiment, pembrolizumab may be administered intravenously.

[39] According to an embodiment, a method for treating a HPV-induced cancer in a subject by combining two distinct treatments for administration to the subject within a common time period of at least 13 weeks is disclosed, wherein the method comprises a HPV vaccine therapy and a checkpoint inhibitor antibody therapy, wherein a checkpoint inhibitor antibody is administered multiple times at a first fixed dose and a vaccine is administered multiple times at a second fixed dose; and wherein a first administration of the HPV vaccine and a fist administration of the checkpoint inhibitor are occurred on day of the at least 13 weeks period, and subsequent administrations of the HPV vaccine and subsequent administration of the checkpoint inhibitor are occurred within the common time period.

[40] In an aspect of the combination treatment, the first fixed dose of the checkpoint inhibitor agent is 50 mg to 500 mg and the second fixed dose of the HPV vaccine is 0.5 mg to 5 mg. According to the method, the HPV vaccine is administered intramuscularly and the checkpoint inhibitor is administered intravenously. In an embodiment, the checkpoint inhibitor is an anti-PD1 antibody or anti-PD-L1 antibody, and wherein the HPV vaccine comprises a nucleic acid construct of SEQ ID NO: 15 or a functional variant with sequence identity of 85% or more to SEQ ID NO: 15. In still another embodiment, the HPV-induced cancer is metastatic, recurrent or advanced cervical cancer and the subject has been or is subject to an anti-cancer treatment; the HPV is HPV 16, HPV 18, or a combination thereof; and the subject is PD-L1 positive or PD-L1 negative. In another embodiment, the individual is PD-L1 positive and infected with HPV 16. In still another embodiment, the individual may be PL-L1 positive and infected with HPV 16, and suffers from squamous cell carcinoma.

[41]

Brief Description of Drawings

[42] FIG. 1 is a schematic illustration of the structure of a vector carrying GX-188.

[43] FIG. 2A illustrates a schedule of administrations of tested drug(s) and evaluations of anti-tumor effects in cervical cancer animal model using C57BL/6 mice. D0, D7, D14, D21, D28, D35, D42, and D49 indicate the number of days from the start (D0). Test drugs include anti-mPD1 monoclonal antibody (mAb), GX-188E, and a GX-188E + anti-mPD1 mAb. TC-1/Luc, which is a cell line from C57BL/6 mouse lung epithelial cells transformed to express E7 of HPV type 16, is used to induce tumor in C57BL/6 mice, as described in Experiment 1.

[44] FIGS. 2B - 2E show anti-tumor efficacy by control (FIG. 2B), anti-mPD1 mAb alone (FIG. 2C), GX-188E alone (FIG. 2D), and a combination of anti-mPD1 mAb + GX-188E (FIG. 2E). GX-188E alone administration group showed a distinct increase in the mean survival period due to retarded tumor cell growth, while the final survival rate

was 17% (1/6). GX-188E + anti-mPD-1 mAb combination group showed a distinct increase in the retardation of tumor cell growth and survival period, while the survival rate also increased to 50% (3/6).

[45] FIG. 3 shows patient enrolment eligibility and primary objectives and secondary objectives. 54 patients were enrolled and 15 patients are ongoing with treatment. For safety analysis, 54 patients receiving at least one dose of either GX-188E or anti-mPD1 mAb (pembrolizumab) were included. For efficacy analysis, 48 patients receiving at least 45 days of treatment were considered evaluable for response to GX-188E by protocol. This interim analysis was performed after obtaining at least one post baseline tumor assessment data.

[46] FIG. 4 shows baseline characteristics of combinational therapy of GX-188E plus anti-PD1 mAb (pembrolizumab) in comparison with anti-PD1 mAb (pembrolizumab) monotherapy.

[47] FIG. 5 shows safety profile of the GX-188E + anti-PD1 mAb (pemprolizumab) combination treatment, any Grade (31.5), and Grades 3-4 (5.6 %), is comparable to anti-PD1 mAb (pembrolizumab) monotherapy employing Keynote-158 monotherapy, where any Grade (65.3%) & Grades 3-4 (12.2%). One of the 3 patients experiencing grade 3 treatment-related adverse events (TRAEs) also had grade 4. No trial drug-related deaths occurred. Overall, GX-188E combined with anti-PD1 mAb (pembrolizumab) was safe and tolerable.

[48] FIGS. 6A and 6B show Best overall response rate (BORR) assessed by RECIST. Six complete responses (CRs) are confirmed, and all cases of CR are observed in patients with PD-L1 positive, HPV 16+ and squamous cell carcinoma. Clinical response is observed in patients with PD-L1 negative, HPV 18+ or adenocarcinoma.

[49] FIG. 7 shows longitudinal change in target lesion from baseline in tumor burden. Currently, Median follow-up was 6.1 months (range; 1.7 - 24.2 months). CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease.

[50] FIGS. 8 show the duration of response in patients whose best overall response was CR and PR (N=16) and eight of 16 responses were ongoing. Median PFS was 2.7 months (range; 1.3-24.2) and median OS was not reached.

[51] FIGS. 9A-9D show T scan of the target lesion at baseline and post GX-188E with pembrolizumab treatment at weeks 10 and 19 in a patient with CR (FIGS. 9A and 9B), and in a patient with PR (FIGS. 9C and 9D). In FIG. 9A, a 63-year-old cervical cancer patient with HPV 16, PD-L1-positive, squamous cell carcinoma who previously received two lines of chemotherapy and had right hilar lymph node metastasis. Axial lung CT showed metastatic lymphadenopathy (arrow). FIG. 9B shows the level of two tumor markers (CEA and TA4) after the combination treatment; dotted lines indicate the cutoff criteria for normal level of tumor markers. In FIG. 9C, a 41-year-old cervical

cancer patient with HPV 18, PD-L1-positive, adenocarcinoma who received two lines of chemotherapy. Pelvis CT revealed ova, low-density metastatic mass (arrow) in the pelvic cavity. FIG. 9D shows the level of two tumor markers (CEA and TA4) after the combination treatment; dotted lines indicate the cutoff criteria for normal level of tumor markers.

[52] FIG. 10 is a graph showing maximum changes from baseline in sum of target lesion size. Maximum changes from baseline in target lesion were assessed by RECIST v1.1 in patients with one or more evaluable post-baseline images (n=45). Each bar represents one patient and dotted lines indicate RECIST v1.1 criteria for PD (+20%) or PR (-30%). Among 48 patients, 3 patients (patient ID 1607, 1608, 1802) were not included in this graph because target lesion were assessed as not evaluable by blinded independent central review (BICR).

[53]

Best Mode for Carrying out the Invention

[54] **DEFINITION OF TERMS**

[55] Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. Unless explicitly stated otherwise, or apparent from context, the terms and phrases below do not exclude the meaning that the term or phrase has acquired in the art to which it pertains. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the aspects provided herein, because the scope of the aspects provided herein is limited only by the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which aspects provided herein belong.

[56] The term "GX-188 variant," "GX-188 analogue," "GX-188 variant construct," "GX-188 analogue construct" or any similar terms as used herein indicate that the construct, after administration of at least one dose of the construct, induces a cellular immune response *in vivo* similar to the cellular immune response induced after administration of GX-188 (FIG. 1 or SEQ ID NO: 9). The cellular immune response can be similar if the variant construct can induce a cellular immune response the same as or higher than the cellular immune response induced by GX-188. In other embodiments, the cellular immune response can be similar if the variant construct induces a cellular immune response at least about 0.9 fold (e.g., 90%), about 0.8 fold, about 0.7 fold, about 0.6 fold, about 0.5 fold, or about 0.4 fold higher than the immune response induced by GX-188. In one embodiment, the cellular immune response is a CD8 T cell response, CD4 T cell response, cytokine secretion, or any combination thereof. In another embodiment, the cellular immune response comprises an increased number of poly-

functional T cells. In certain embodiments, the poly-functional T cells exhibit at least three, at least four, or at least five markers selected from the group consisting of IFN-γ, IL-2, TNF-α, MIP-β, CD107a/b, and any combination thereof, when measured by flow cytometry. An example of the GX-188 variant may be GX-188E (SEQ ID NO: 15).

[57] As used herein, the term "GX-188E" is the nucleic acid construct having the nucleotide sequence of SEQ ID NO: 15 or its variant having sequence identify of at least about 80% or more, about 81% or more, about 82% or more, about 84% or more, about 85% or more, about 86% or more, about 87% or more, about 88% or more, about 89% or more, about 90% or more, about 91% or more, about 92% or more, about 93% or more, about 94% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, about 99% or more, or about 100% to SEQ ID NO: 15.

[58] The term "sequence identity" between two polypeptides is determined by comparing the amino acid sequence of one polypeptide to the sequence of a second polypeptide. When discussed herein, whether any particular polypeptide is at least about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). BESTFIT uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full-length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[59] The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower).

[60] The term "significant" or "significantly" refers to statistical significance and generally means a two standard deviation (2SD) or greater difference.

[61] As used herein the term "comprising" or "comprises" is used in reference to com-

positions, methods, and respective component(s) thereof, that are essential to the method or composition, yet open to the inclusion of unspecified elements, whether essential or not.

[62] The term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[63] As used herein the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment.

[64] The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The abbreviation, "e.g." is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example".

[65] In an embodiment, the "immunomodulatory compound" includes but is not limited to cytokines, such as interferons, monoclonal antibodies, such as a PD-1/PD-L1 pathway inhibitor (PD-1/PD-L1 inhibitor), anti-CTLA4 antibodies, cyclophosphamide, Thalidomide, Levamisole, Lenalidomide, or a combination thereof. In an embodiment, the immunomodulatory compound is an anti-PD1 antibody, anti-PD-L1 antibody, anti-CTLA4 antibody, a combination of anti-PD1 antibody and an anti-CTLA4 antibody, a combination of anti-PD-L1 antibody and an anti-CTLA antibody. The term "PD-1/PD-L1 pathway inhibitor" is a compound inhibits or blocks a binding of PD-L1 to PD-1 and may include an anti-PD-1 antibody, an anti-PD-L1 antibody, and an anti-PD-1/PD-L1 antibody.

[66] The immunomodulatory compounds may be selected from an anti-PD1 antibody or an anti-PD-L1 antibody such as pembrolizumab or MDX-1106 (Merck), nivolumab, cemiplimab, atezolizumab, avelumab, durvalumab, THALOMID® (thalidomide), cyclophosphamide, Levamisole, lenalidomide, CC-4047 (pomalidomide), CC-11006 (Celgene), and CC-10015 (Celgene), and immunomodulatory compound described in any one of WO2007028047, WO2002059106, and WO2002094180. The immunomodulatory compound may an anti-PD1 antibody. In an embodiment, the anti-PD1 antibody is pembrolizumab.

[67] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma,

blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancers.

[68] The terms "tumor" and "neoplasm" refer to any mass of tissue that result from excessive cell growth or proliferation, either benign (noncancerous) or malignant (cancerous) including pre-cancerous lesions. Tumor can be a cervical tumor. In specific embodiments, the cervical tumor is a benign tumor or a malignant tumor. In certain embodiments, the cervical tumor is squamous cell carcinoma (SCC), adenocarcinoma, adenosquamous carcinoma, small cell carcinoma, neuroendocrine tumor (NET), glassy cell carcinoma, villoglandular adenocarcinoma (VGA), non-carcinoma malignancies, melanoma, lymphoma, or cervical intraepithelial neoplasia (CIN). In some embodiments, the cervical tumor is CIN1, CIN2, CIN3, or cervical cancer.

[69] The terms "cancer cell," "tumor cell," and grammatical equivalents refer to the total population of cells derived from a tumor or a pre-cancerous lesion, including both non-tumorigenic cells, which comprise the bulk of the tumor cell population, and tumorigenic stem cells (cancer stem cells).

[70] An "effective amount" of a polynucleotide encoding a fusion protein as disclosed herein is an amount sufficient to carry out a specifically stated purpose. An "effective amount" can be determined empirically and in a routine manner, in relation to the stated purpose.

[71] As used herein, a "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutic result may be, e.g., lessening of symptoms, prolonged survival, reduction in size and/or volume of tumor, inhibition of growth of tumor, and the like. A therapeutic result need not be a "cure".

[72] The terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, halt progression of a diagnosed pathologic condition or disorder, reduce in size or volume of tumor tissue, and/or stop of tumor growth. Thus, the subjects in need of treatment include those already diagnosed with or suspected of having the disorder.

[73] By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include, but are not limited to, humans, domestic

animals, farm animals, zoo animals, sport animals, pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows; primates such as apes, monkeys, orangutans, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equids such as horses, donkeys, and zebras; bears, food animals such as cows, pigs, and sheep; ungulates such as deer and giraffes; rodents such as mice, rats, hamsters and guinea pigs; and so on. In certain embodiments, the mammal is a human subject.

[74] The term "combination treatment/therapy," "combined treatment," "combinatorial" or "in combination" means at least a vaccine and checkpoint inhibitor treatment, at the same time and/or at different times, within a prescribed time period, with at least the said two distinct therapeutic agents.

[75] The term "checkpoint inhibitor" and/or "antibody" means any one or more of commercial drugs and/or non-commercial drugs designed, whether or not commercialized and/or sold to administer to an individual (or an animal), for unblocking checkpoints in the body which may prevent the immune system, in part or in whole, from attacking a cancer using the body's T cells, and regardless of how administered.

[76] The term "PD-1" means one example of a checkpoint inhibitor antibody.

[77] The term "baseline" means the tumor volume (TV) at day 1 for Experiments.

[78] The term "vector" is a term that contains a transcription unit (also known as the "expression vector") and as used herein refers to a viral and/or non-viral expression vector that when administered in vivo can enter target cells and express an encoded protein. Viral vectors suitable for delivery in vivo and expression of an exogenous protein are well known and include adenoviral vectors, adeno-associated viral vectors, retroviral vectors, vaccinia vectors, pox vectors, herpes simplex viral vectors, and the like. Viral vectors are preferably made replication defective in normal cells. For example, see U.S. Pat. Nos. 6,669,942; 6,566,128; 6,794,188; 6,110,744 and 5,133,029. The vector can be administered parenterally, such as intravenously, intra-arterially, intramuscularly, subcutaneously, or the like. Administration can also be orally, nasally, rectally, trans-dermally or aerosol inhalation. The vectors may be administered as a bolus or slowly infused. The vector in the instant application is preferably administered subcutaneously.

[79]

[80] **COMPOSITION AND TREATMENT**

[81] The composition of GX-188 may be administered parenterally, by injection, for example, either subcutaneously, intracutaneously, intradermally, subdermally or intramuscularly. The terms "GX-188 composition," "composition of GX-188," "GX-188 formulation" and "GX-188-containing formulation" as used herein refers to a composition or formulation comprising a polynucleotide construct comprising the sequence

of SEQ ID NO: 9 or 15 or a variant thereof with sequence identity of about 85% or more, about 86% or more, about 87% or more, about 88% or more, about 89% or more, about 89% or more, about 90% or more, about 91% or more, about 92% or more, about 93% or more, about 94% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more to SEQ ID NO: 9 or SEQ ID NO: 15.

[82] Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral, nasal, buccal, sublingual, intraperitoneal, intravaginal, anal, epidural, spinal, and intracranial formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10% (w/w), preferably 1% to 2% (w/w). Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and may contain 10% to 95% (w/w) of active ingredient, preferably 25% to 70% (w/w).

[83] The GX-188-containing formulation may be administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including, e.g., the capacity of the individual's immune system to mount an immune response, and the degree of immunity desired. Suitable dosage ranges are of the order of several hundred micrograms of active ingredient per vaccination with a preferred range from about 1 μg to 20 mg, such as in the range from about 5 μg to 10 mg. In an embodiment, the dose may range from about 0.5μg to 1000μg, 1μg to 1000 μg, or in the range from 1 μg to 500 μg and especially in the range from about 10 μg to 100 μg. In another aspect, the dosage may be in a range from 0.1 mg to 20 mg. In another aspect, the dosage may range from 0.5 mg to 10 mg. In still another aspect, the dosage may range from 1 mg to 5 mg. In still another aspect, the dosage may be in ranges of 0.5 mg to 20mg, 1 mg to 20 mg, 0.5 mg to 5 mg, 1.5 mg to 10 mg, 2 mg to 5 mg, 2.5 mg to 5 mg, 3 mg to 5 mg, or 2 mg to 10 mg. In another embodiments, the dosage of GX-188 may be about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.6 mg, about 1.7 mg, about 1.8 mg, about 1.9 mg, about 2 mg, about 2.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.6 mg, about 1.7 mg, about 1.8 mg, about 1.9 mg, about 2 mg, about 2.1 mg, about 2.2 mg, about 2.3 mg, about 2.4 mg, about 2.5 mg, about 2.6 mg, about 2.7 mg, about 2.8 mg, about 2.9 mg, about 3 mg, about 3.1 mg, about 3.2 mg, about 3.3 mg,

about 3.4 mg, about 3.5 mg, about 3.6 mg, about 3.7 mg, about 3.8 mg, about 3.9 mg, about 4 mg, about 4.1 mg, about 4.2 mg, about 4.3 mg, about 4.4 mg, about 4.5 mg, about 4.6 mg, about 4.7 mg, about 4.8 mg, about 4.9 mg, or about 5 mg, administered in at intervals of about every week, once every other week, once every three weeks, once every four weeks. The GX-188 formulation may be administered at an interval of 5 days, 10 days, 15 days, 20 days, or 30 days, or 40 days.

[84] According to an aspect, suitable regimens for initial administration and booster shots are also variable but are typified by an initial administration followed by subsequent inoculations or other administrations. For example, in one embodiment, the GX-188 may be administered at a fixed dosage of about 2 mg at week 1, week 2, week 4, week 7, week 13, and week 19, and optionally further at week 46. The administration may be made via an intramuscular route. In another embodiment, GX-188 may be administered at a fixed dosage of about 2 mg at week 1, week 2, week 4, week 8, week 14, and week 20, and optionally further at week 46. In other embodiments, the dosage could vary at each administration.

[85] The immunomodulatory agent may be administered as a separate formulation. In an embodiment, an anti-PD1 antibody may be administered intravenously simultaneously or at different time from the GX-188 administration. The anti-PD1 antibody may be administered at a dose of about 1 mg to 1000 mg. In an embodiment, the dose of anti-PD1 antibody may be in ranges of about 10 mg to 500 mg, about 50 mg to 500 mg, about 100 mg to 500 mg, abut 100 mg to 300 mg, about 150 mg to 300 mg, about 180 mg to 250 mg, about 190 mg to 250 mg, about 185 mg to 225 mg, about 185 mg to 220 mg, about 195 mg to 250 mg, about 195 mg to 225 mg, about 190 mg to 230 mg, about 200 mg to 400 mg, about 200 mg to 300 mg, about 250 mg to 300 mg, about 280 mg to 350 mg, about 300 mg to 500 mg, about 300 mg to 400 mg, about 300 mg to 1000 mg, about 300 mg to 900 mg, about 300 mg to 800 mg, about 300 mg 700 mg, or about 300 mg to 600 mg. The anti-PD1 antibody may be administered at intervals of once per week, twice a week, three times a week, four times a week, every week, every 10 days, once every other week, every 20 days, every three weeks, every four weeks, once a month, once every other month, or once every three months, etc. In an embodiment, anti-PD1 antibody is pembrolizumab and may be administered at a dose of 200 mg intravenously three times a week, starting before, at the same time, or after the first administration of GX-188 for the duration of 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks or longer. The duration may be measured by calendar months such as for 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, or longer. Or, the treatment regimen may include the number of administrations such as 10 doses, 11

doses, 12 doses, 13 doses, 14 doses, 15 doses, 16 doses, 17 doses, 18 doses, 19 doses, 20 doses, 21 doses, 22 doses, 23 doses, 24 doses, 25 doses, 26 doses, 27 doses, 28 doses, 29 doses, 30 doses, 31 doses, 32 doses, 33 doses, 34 doses, 35 doses, or more, at a fixed dosage of, for example, about 200 mg.

[86] A fusion protein comprising the sequence of SEQ ID NO: 10 or its functional variant may be formulated into a vaccine as neutral or salt forms. Pharmaceutically acceptable salts include acid addition salts (formed with the free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and organic bases such as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like. A functional variant of fusion protein comprising the sequence of SEQ ID NO: 10 may be a polypeptide of sequence identity of about 85% or more, about 86% or more, about 87% or more, about 88% or more, about 89% or more, about 89% or more, about 90% or more, about 91% or more, about 92% or more, about 93% or more, about 94% or more, about 95% or more, about 96% or more, about 97% or more, about 98% or more, or about 99% or more to SEQ ID NO: 10.

[87] According to an embodiment, the administration of GX-188 and anti-PD1 antibody to a patient with an advanced, inoperable, or metastatic cervical cancer, shows significant treatment effects such as decrease in the size and/or volume of tumor, and improved anti-cancer immune responses as measured by T cell response, PD-L1, CEA, and/or TA4 levels. For example, the administration of a GX-188 variant and an anti-PD1 antibody effectively induced HPV-specific T cell responses. Whereas pembraolizumab monotherapy has no effects on PD-1 negative patients, the combination therapy of GX-188 and pembraolizumab is effective in treating or enhancing treatment of cervical cancer patient who is PD1 negative.

[88]

Mode for the Invention

[89] Hereinafter, the present invention will be described in more detail with reference to Examples. It will be apparent to those skilled in the art that the following Examples are merely illustrative of the present invention and that the scope of the present invention is not limited by these Examples.

[90]

[91] **Example 1. Pre-Clinical Study**

[92] Anti-tumor efficacy of a combination therapy with GX-188E and anti-PD1 antibody

on cervical cancer was assessed in an animal model. TC-1 cells, which are a cell line from C57BL/6 mouse lung epithelial cells transformed to express E7 of HPV type 16, were selected as the cell line for the production of an animal model for GX-188E efficacy assessments.

[93] After tumor formation by the subcutaneous transplantation of 1 X 10^5 TC-1 tumor cells (provided by Professor Jaetae Lee, Kyungpook National University Hospital) in C57BL/6 mice, the mice were intramuscularly administered 4 μg of GX-188E or formulation buffer (PBS) via electroporation (OrbijectorTM, Elimtek Co., Ltd) at weeks 1, 2, and 4. For the combination therapy and anti-mouse PD-1 antibody (anti-mPD-1 mAb) alone groups, 250 μg anti-mPD1 antibodies (BioXcell, clone RMP1-14) were intraperitoneally administered five times at 1-week intervals, starting 2 weeks after the transplantation of TC-1 cells. After the tumor cell challenge, tumor size was measured and survival rate was checked twice a week (FIG. 2A).

[94] All animals in the control (PBS) group died, which confirmed that the tumor challenge conditions used were appropriate. When only anti-mPD-1 antibody was administered, some animals showed a weak effect, i.e. a delayed tumor cell growth, compared to that in the control group. However, because all animals eventually died, the anti-mPD-1 antibody did not induce any change in survival rate. When only GX-188E was administered, there was a distinct increase in the mean survival period due to retarded tumor cell growth, while the final survival rate was 17% (1/6). On the other hand, when a combination of GX-188E and the anti-mPD-1 antibody was administered, there was a distinct increase in the retardation of tumor cell growth and survival period, while the survival rate also increased to 50% (3/6) FIGS. 2B-2E).

[95] Based on the findings, the inventors determined that the antigen-specific cellular immune response induced by GX-188E was able to effectively remove tumor cells that had already formed, which lead to delayed tumor cell growth and an increased survival period. Moreover, the group that received the combination of an immunomodulatory compound and GX-188E therapeutic vaccine showed similar levels of delayed tumor cell growth and prolonged survival as the GX-188E alone group, which also lead to an increase in the survival rate from a long-term perspective.

[96] Inferring based on the mechanism of action of the given checkpoint inhibitor, the findings of this study were similar to results of previous studies, which indicated that the anti-PD1 antibody prolonged the anti-tumor killing activities of T cells. Accordingly, the findings suggest that a checkpoint inhibitor is a suitable synergistic partner for enhancing the efficacy of a HPV-targeted immunotherapeutic vaccine including GX-188E.

[97] The findings of this study showed that combining an anti-PD1 antibody with the immunotherapeutic vaccine GX-188E enhanced the efficacy of GX-188E, which

suggested that a checkpoint inhibitor could be a suitable candidate for use in combination therapy with an immunotherapeutic vaccine.

[98]

[99] **Example 2: Clinical Study**

[100] GX-188E (SEQ ID NO: 15) is an HPV therapeutic DNA vaccine encoding HPV 16/18 E6/E7. Twelve (12) precancer patients were immunized with GX-188E, and seven of nine patients in phase I and 35 of 52 patients in phase II presented regression of cervical lesion within 36 weeks after vaccination. The clinical benefits were associated with enhanced HPV specific IFN-γ responses by GX-188E vaccination. Given the clinical proof of concept in precancer patients, the inventors speculated that, in cervical cancer patients, GX-188E vaccination increases the proportion of clinical responders to a checkpoint inhibitor by increasing the frequency of HPV-specific T cells.

[101] In phase II clinical trial, the inventors evaluated the safety and efficacy of GX-188E combined with pembrolizumab in patients with HPV 16 and/or 18-positive recurrent/advanced cervical cancer who failed the first or later lines of chemotherapy.

[102]

[103] **A. Study design and participants**

[104] The study was a prospective, open-label, phase II study. The protocol was approved by the institutional review board or ethics committee at each study site, and a written informed consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki and all applicable laws.

[105] Fifty-four patients were enrolled and treated with the investigational combination. The inclusion criteria included female patients aged ≥ 18 years who signed an informed consent; those who presented with Eastern Cooperative Oncology Group performance status of 0-1 and histologically confirmed recurrent/advanced HPV-positive (HPV 16 and/or HPV 18) cervical cancer; those who had disease progression after treatment with available therapies for recurrent/advanced cancer. Patients were excluded from the study if they had a history of active central nervous system metastases or active autoimmune disease, an allogeneic solid organ or bone marrow transplant, or a diagnosis of immunodeficiency. All patients submitted either an archival or fresh biopsy sample of their tumor for molecular and histological analyses at screening/baseline. Peripheral blood samples were taken from the patients at screening and at week 1, 4, 7, 10, 16, 22, and 49 for IFN-γ ELISpot assays.

[106]

[107] **B. Procedures**

[108] GX-188E was administered intramuscularly 2 mg either into deltoid or lateralis muscles, followed immediately by co-localized electroporation (TriGrid Delivery System, Ichor medical systems, Inc.) at weeks 1, 2, 4, 7, 13, and 19 with one optional

dose at week 46. Pembrolizumab was administered using an intravenous infusion on day 1 of each 3-week treatment cycle after all procedures and assessments have been completed, based on the standard clinical and institutional practices.

[109] To investigate the cellular immune response induced by GX-188E, HPV 16/18 E6/E7-specific T cell responses were analyzed at indicated time in 'Study design and participants'. For the IFN-γ ELISpot analysis (BD Bioscience, CA, USA), cryopreserved and thawed peripheral blood mononuclear cells (PBMCs) were adapted and further processed as described previously in Kim TJ, Jin H-T, Hur S-Y, et al. Clearance of persistent HPV infection and cervical lesion by therapeutic DNA vaccine in CIN3 patients. Nature communications 2014; 5(1): 1-14. T cell responses to HPV E6/E7 were measured by comparing signals to the baseline levels and considered positive when the response after vaccination was five-fold higher than that at baseline.

[110]

[111] **C. Outcomes**

[112] Patients were radiographically assessed for responses (by both RECIST v1·1 and iRECIST) approximately every 9 weeks. The safety of the investigational product was evaluated by recording, reporting, and analyzing the results of the laboratory tests and physical examination findings, which considered the patient's underlying disease, adverse reactions, and vital signs. The adverse events (AEs) experienced by patients, such as drug toxicity, was comprehensively evaluated. The investigators evaluated the severity of AEs based on the Common Terminology Criteria for Adverse Events (CTCAE v4·03) by the National Cancer Institute.

[113]

[114] **D. Statistical analysis**

[115] All patients who received at least one dose of the investigational treatment were included in the safety population and analyzed for safety profile. AEs were coded according to the MedDRA adverse event dictionary. The results were tabulated to examine their frequency, the organ systems they affected, and their relationship to the study treatment. Efficacy results, including Best overall response rate, were analyzed using descriptive statistics. Objective responses were evaluated according to both RECIST v1·1 and Response Evaluation Criteria in Solid Tumors for Immunotherapeutics (iRECIST). Confirmatory scans were acquired for all determinations of objective response (PR or CR), stable disease (SD), and disease progression (PD).

[116]

[117] **E. Results**

[118] 54 patients were enrolled and 48 patients were evaluated for safety (FIG. 3). Three patients not receiving 45 days of treatment were considered non-eligible for evaluation of response to GX-188E vaccination by protocol. Therefore, 48 patients were

evaluated for treatment efficacy. The baseline characteristics of the patients who received GX-188E and pembrolizumab combination are shown in FIG. 4, and were similar to those treated with previously reported pembrolizumab monotherapy, except histology and HPV types.

[119] Fifty-four (54) patients were treated and evaluated for safety, showing 31.5% TRAEs (Treatment related Adverse Events) of any grade and 5.6 of grade 3-4 TRAEs (FIG. 5). The most common TRAEs, as classified by the systemic organ class, were gastrointestinal disorders (9.3%) and skin and subcutaneous tissue disorders (7.4%). Three patients (5.6%) experienced grade 3 TRAEs, including one patient with a grade 3 elevation of aspartate aminotransferase (AST) level associated with a grade 4 elevation of alanine aminotransferase (ALT) level. This patient discontinued treatment owing to TRAEs, which were assessed as immune-related adverse events (irAEs). Overall, GX-188E vaccination in combination with an anti-PD1 antibody (pembrolizumab) administration was considered safe and tolerable. FIGS. 6A and 6B show a summary of antitumor responses assessed by radiologists at the study sites.

[120]

[121] In FIGS. 5, 6A, and 6B, abbreviations stand for the following meanings:

[122] TRAE: Treatment related Adverse Event;

[123] BOR: Best Overall Response;

[124] DOR: Duration of Response;

[125] CR: Complete Response;

[126] PR: Partial Response;

[127] SD: Stable Disease;

[128] PD: Progressive Disease;

[129] NE: Non evaluable;

[130] DCR: Disease Control Rate;

[131] PD-L1: programmed death-ligand 1;

[132] SCC: Squamous Cell Carcinoma; and

[133] AC: Adenocarcinoma.

[134]

[135] As seen in FIG 6A, BORR 33.3% (16/48) and DCR was 50% (24/48). Among 16 patients with BORR, six patients were confirmed to have CR, which was all durable and ongoing, with duration of response ranging from 1.3 to 24.2 months at the cutoff time; all CR patients were PD-L1 positive, and with HPV 16 and squamous cell carcinoma. Responses were observed in both HPV 16- and/or 18-positive patients, although HPV 16-positive showed favorable response (35.3% vs. 28.6%). Unexpectedly, the response was observed not only in PD-L1-positive tumor but also in PD-L1-negative tumor: the BORR and DCR were 41.7% (15/36) and 61.1% (22/36) in

patients with PD-L1-positive tumor and 8.3% (1/12) and 16.7% (2/12) in PD-L1-negative tumor, respectively.

[136] As shown in FIG. 7 and FIG. 8, the patients who showed tumor reduction at week 10 tended to have their responses improved over time. The median follow-up is 6.1 months (range, 1.7 to 24.2 months at cutoff date), by which time 24 patients (50.0%) developed progressive disease (PD).

[137] Images of tumor from a 63-year-old patient are shown in FIG. 9A. The patient had HPV 16- and PD-L1-positive squamous cell carcinoma and previously received two lines of chemotherapy and had lymph node metastasis. Following the treatment, the patient showed CR and a significant decrease to normal level in the tumor marker TA4. FIG. 9C shows images of tumor from a 41-year-old patient with HPV 18- and PD-L1-positive adenocarcinoma who previously received two lines of chemotherapy and had pelvic mass and lymph node metastasis. Following treatment, the patient showed PR and a significant decrease in another tumor marker CEA over time. FIG. 9B and 9D show the level of two tumor markers (CEA and TA4) after the combination treatment; dotted lines indicate the cutoff criteria for normal level of tumor markers.

[138] In FIG. 10, maximum changes from the baseline (shown in FIG. 7) in target lesion size are summarized in a waterfall plot with PD-L1 expression status. PD-L1-positive patients responded better to the combination therapy of GX-188E plus pembrolizumab than did PD-L1-negative patients in terms of BORR (15/36 vs. 1/12) (FIG. 6A).

[139] Importantly, target size reduction higher than 30% was observed in one PD-L1-negative patients whereas no responses were observed in PD-L1-negative patients in the previous study of pembrolizumab alone. Regarding antigen-specific T cell responses, results of IFN-γ ELISpot assay were shown as fold-change from the baseline (not shown). Eighteen of 23 response-evaluable patients (78.3%) showed DNA vaccine-induced T cell responses, indicating that GX-188E in combination with pembrolizumab effectively induced HPV E6/E7-specific T cell responses even in heavily pretreated cancer patients.

[140]

[141] **A. Discussion**

[142] The clinical study results show that an HPV vaccination combined with pembrolizumab induced effective antitumor responses in recurrent/advanced, inoperable, or metastatic cervical cancer patients. The clinical benefit was promising (33.3% BORR in total patients, and 41.7% BORR in PD-L1-positive patients). To our knowledge, this is the first report of a combination therapy with a cancer vaccine and a checkpoint inhibitor for recurrent/advanced cervical cancer patients. Once tumor antigen-specific T cells are induced by a cancer vaccine, they migrate to and infiltrate tumor tissue, converting cold to hot tumor. Tumor-infiltrating lymphocytes (TIL) secrets IFN-γ

which in turn induces expression of PD-L1 from tumor cells, as an adaptive resistance mechanism against immune attack. As a checkpoint inhibitor induces antitumor responses by reinvigorating the tumor cytolytic function of the exhausted TIL, the presence of tumor-specific TIL is a prerequisite for antitumor responses to a checkpoint inhibitor. As evidenced by the correlation of the absence of TIL and PD-L1 negativity with relatively poor clinical response to the checkpoint inhibitor, it is suggested that non-immunogenic cold tumors with PD-L1 negativity requires additional strategies for increasing TIL and PD-L1 expression.

[143] Cancer vaccines are regarded as the most efficient method to induce tumor-specific T cell responses. GX-188E vaccination effectively induced Ag-specific T cell responses in HPV precancer patients. It is likely that GX188E vaccination increases the frequency of TIL, followed by enhanced PD-L1 expression in tumor. This is a possible explanation about how GX188E vaccination enhanced clinical efficacy in combination with an anti-PD1 antibody.

[144] The results show that compared with HPV 16 infection, PD-L1 positive, and squamous cell carcinoma, recurrent/advanced cervical cancer with HPV 18 infection, PD-L1 negative, and adenocarcinoma tends to result in poor clinical responses.

[145] In summary, a combined treatment with GX-188E and a checkpoint inhibitor agent (a PD-1/PD-L1 pathway inhibitor including pembrolizumab) in patients with heavily pretreated recurrent/advanced cervical cancer was safe and tolerable, showing similar safety profile to the previously reported pembrolizumab monotherapy. This result indicated that GX-188E vaccination did not add any significant adverse effects while it effectively induced HPV-specific T cell responses in heavily pretreated cervical cancer patients. The present combination treatment showed high response rate of approximately 41.7 in PD-L1-positive patients, 35.3% in HPV 16 positive patients, and 33.3% in squamous cell carcinoma. And the present combination treatment was effective in PD-L1-negative patients, as opposed to the pembrolizumab monotherapy was not effective. Furthermore, the present combination treatment demonstrated clinical responses also in HPV 18 and adenocarcinoma.

[146] Therefore, the present combination treatment of employing GX-188E combined with an anti-PD1 antibody was safe and efficacious for the treatment of patients with HPV 16-/18-positive recurrent or advanced cervical cancer who failed currently available standard therapies, and has a potential to be a new standard therapy for HPV 16/18-related cancers, such as oropharyngeal and anogenital cancers as well as cervical cancer.