MATERIALS AND METHODS FOR TREATMENT OF MELANOMAS AND OTHER CANCERS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to U.S. Provisional Application No. 63/571,061, filed March 28, 2024, the contents of which are incorporated herein by reference in their entireties.

SEQUENCE LISTING

The Sequence Listing for this application is labeled "SeqList-28Mar24.xml" which was created on March 28, 2024, and is 10 KB. The entire content of the sequence listing is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present disclosure relates generally to DNA vaccine compositions and methods of use thereof to enhance an immune response against cancer, and in particular to methods of enhancing an immune response to immune checkpoint inhibitor resistant cancers and rendering the cancer susceptible to immune checkpoint inhibitor therapies.

BACKGROUND OF THE INVENTION

Multiple treatment modalities are available for treating cancer. These treatments include surgical resection of the tumor, chemotherapy, radio therapies and combination therapies. While resection can be curative, tumors recur in most cases. Chemotherapies utilize drugs that kill tumor cells by intercalation of DNA, inhibition of replication, or prevention of microtubule assembly. To avoid killing healthy cells, a balance must be achieved by fine tuning the chemotherapy doses and regimens, which should be based on the type of tumor, stage, grade, and overall tumor burden. Radiotherapy is essentially geared to kill cancer cells by damaging DNA. Both chemo and radiotherapies result in a number of side effects in patients, including resistance to therapies, and in most cases, tumors recur.

Conventional treatments for cancers typically include chemotherapy and/or surgery. Recently there has been interest in developing vaccines in an effort to stimulate an immune defense.

Tumor antigen vaccines generally show poor antigenicity due to immune tolerance. The adaptive immune response to tumors alone is poor, mostly because the target antigens are self-proteins, except in cases of tumor induced by viruses. Most require interventional

therapies in order to provide an adequate "danger" signal to the immune system in order to activate a robust, clinically-meaningful antitumor immunity.

While whole cell vaccines have been demonstrated to have clinical activity, manufacturing this type of vaccine requires surgical removal of patient's palpable lymph node, ex vivo processing of cells, transformation of cells with Emm protein, irradiation of tumor cells, and the quality control (QC) of the vaccine for individual patients. With solid tumors, it can be difficult to obtain sufficient cells for processing, and processing can require from one to several weeks.

Another approach to cancer vaccines has been to mix cell lines of a selected type of cancer derived from different individuals of the same cancer. An allogeneic pancreatic cancer cell line expressing the adjuvant GM-CSF was used in a phase I clinical trial following pancreaticoduodenoectomy. The administered cells proved to be non-toxic but delayed type hypersensitivity (DTH) reaction in the recipients was observed (Jaffe, EM et al., J. Clin. Oncol. 2001, 19, 145–156).

Plasmid DNA vaccines are circular DNA encoding one or more tumor associated antigens (TAAs) and immune-stimulating or co-stimulating molecules, which are administered intramuscularly, intranodally or intratumorally. The local tissue specific cells at the injection site then express the antigens to stimulate the immune system.

Checkpoint inhibitors (CPIs), or immune checkpoint inhibitors, are a normal part of the immune system. Their role is to prevent an immune response from being so strong that it destroys healthy cells in the body. Immune checkpoints engage when proteins on the surface of the immune cells, T cells, recognize and bind to partner proteins on other cells, such as, for example tumor cells. When the checkpoint and partner proteins bind, they send an immunosuppressive signal to the T cells, which can prevent the immune system from destroying cancer. Checkpoint inhibitors are typically antibodies or siRNAs that can be developed and used to revert immune exhausted T cells into activated T cells within the tumor microenvironment.

Immune checkpoint inhibitor drugs that are currently used in cancer treatment include checkpoint proteins called CTLA-4, PD-1, and its partner protein PD-L1. CTLA-4 is a checkpoint protein on some T cells that acts as an off switch to keep the immune system in check. Monoclonal antibody treatments attach to CTLA-4 and stop it from working, which can help boost the body's immune system. PD-1 and PD-L1 inhibitors bind on cells

and send a signal to the T cells to leave the other cells alone. Some cancer cells have large amounts of PD-L1 which can hide them from an immune attack.

CPIs can have serious side effects and are not always able to successfully treat cancer due to the cells developing resistances to them. Negative side effects can include diarrhea, fatigue, cough, nausea, skin rash, poor appetite, constipation, muscle and joint pain, infusion reactions, and autoimmune reactions. CPIs remove one of the safeguards on the body's immune system. Sometimes the immune system can respond to these treatments by attacking various parts of the body, which can cause serious/life threatening problems.

Resistance can occur when cancer cells contain molecular changes that make them intensive to a particular drug before treatment begins. Because cancer cells within the same tumor often have a variety of molecular changes, resistance is very common.

Many patients with cancer are treated with checkpoint inhibitors, but still develop disease progress, with either primary or secondary CPI resistance. CPI resistances can include inhibitory or other checkpoints, defects in neoantigen loss and antigen presentation, oncogenic signaling pathways, and immunosuppressive tumor microenvironment. Thus, novel treatments that overcome these resistances are needed to progress the field of cancer treatment.

In 2023, 97,610 cases of invasive melanoma, with 7,990 deaths, were predicted in the United States[1]. The response rate of single agent anti-PD-1 (nivolumab, pembrolizumab) is approximately 40%, and in combination with anti-CTLA-4 or anti-LAG3 therapy response rates increase to 50-60%, at the cost of significantly increased toxicity[2, 3, 4, 5, 6, 7, 8]. Talimogene laherparepvec (TVEC) is an oncolytic live virus approved for intralesional administration into cutaneous, in-transit, or nodal melanoma metastases[9]. TVEC suffers from many limitations, and there remains a major unmet need for new intralesional immunotherapeutic approaches[10].

Melanoma cells may 'hide' tumor-specific antigens from the immune system or take advantage of the normal inhibitory immune pathways designed to protect a person from autoimmunity[22]. The present disclosure utilizes a fragment of an M protein to generate an immune response expressed in melanoma tumors via a plasmid approach such that the body recognizes the tumor as foreign. Due to high variability at the N-terminal region of M proteins, more than 200 distinct types have been recorded in public databases.

SUMMARY OF THE PRESENT TECHNOLOGY

In one aspect, the present disclosure provides an anti-cancer vaccine comprising a therapeutically effective amount of a nucleic acid sequence encoding *emm*55, a cationic polymer, and dextrose. In some embodiments, the nucleic acid sequence encoding *emm*55 comprises SEQ ID NO: 1. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 1μg to about 10,000μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 100μg. In some embodiments, the vaccine further comprises a pharmaceutically acceptable carrier selected from the group consisting of a cream, emulsion, gel, liposome, nanoparticle, or ointment. In some embodiments, the vaccine is formulated for intralesional, pleural, topical, parenteral, intravenous, subcutaneous, intranodal, intratumoral, intrathecal, intrapleural or intraperitoneal administration. In some embodiments, the vaccine is formulated for intralesional administration. In some embodiments, the cationic polymer is selected from the group consisting of poly(ethyleneimine), poly(propylenimine), poly(amido amine), poly(β-amino ester), poly[2-(dimethylamino)ethyl methacrylate], and poly(L-lysine). In some embodiments, the cationic polymer is a linear poly(ethyleneimine) or a branched poly(ethyleneimine). In some embodiments, the vaccine comprises about 50μg to about 250μg cationic polymer. In some embodiments, the vaccine comprises about 144μg cationic polymer. In some embodiments, the vaccine comprises about 1% to about 20% dextrose. In some embodiments, the vaccine comprises about 10% dextrose.

In another aspect, the present disclosure provides a method for treating immune checkpoint inhibitor therapy resistant cancer in a subject in need thereof comprising administering to the subject the vaccine of any one of the preceding embodiments. In some embodiments, the subject had previously been treated with a PD-1 checkpoint inhibitor therapy. In some embodiments, the vaccine is administered intralesionally, pleurally, topically, parenterally, intravenously, subcutaneously, intranodally, intratumorally, intrathecally, intrapleurally, or intraperitoneally. In some embodiments, the vaccine is administered intralesionally. In some embodiments, the method further comprises administering an additional therapeutic agent. In some embodiments, the additional therapeutic agent is administered simultaneously, sequentially, or separately to the vaccine. In some embodiments, the additional therapeutic agent comprises an immune checkpoint inhibitor. In some

embodiments, the immune checkpoint inhibitor comprises one or more of an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-TIM3 antibody, an anti-4-1BB antibody, an anti-CD73 antibody, an anti-GITR antibody, or an anti-LAG-3 antibody. In some embodiments, the immune checkpoint inhibitor comprises an anti-PD-1 antibody and/or an anti-PD-L1 antibody. In some embodiments, the anti-PD1 antibody is selected from the group consisting of Nivolumab, Pembrolizumab, Cemiplimab, Tislelizumab, Dostarlimab, Retifanlimab, and Toripalimab. In some embodiments, the anti-PD-L1 antibody is selected from the group consisting of Atezolizumab, Avelumab, Durvalumab, and Cosibelimab. In some embodiments, the cancer is a solid tumor. In some embodiments, the tumor is a melanoma. In some embodiments, the melanoma is Merkel Cell Carcinoma. In some embodiments, administration of the vaccine increases sensitivity of the cancer to treatment with PD1 checkpoint inhibitor therapy. In some embodiments, the PD1 checkpoint inhibitor therapy is selected from the group consisting of Nivolumab, Pembrolizumab, Cemiplimab, Tislelizumab, Dostarlimab, Retifanlimab, Toripalimab, Atezolizumab, Avelumab, Durvalumab, and Cosibelimab. In some embodiments, administration of the vaccine increases expression of one or more genes selected from the group consisting of CXCL13, CD38, LAG3, STAT1, STAT2, CXCL11, CXCL13, IFI6, MX1, and LAG3. In some embodiments, administration of the vaccine decreases the expression of one or more genes selected from the group consisting of WNT2, WNT4, and PNOC.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a map of the pAc/*emm55* plasmid construct.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 is the nucleotide sequence for pAc/*emm*55. **SEQ ID NO: 2** is the amino acid translation of *emm*55 gene sequence (encoded by SEQ ID NO: 1).

DETAILED DESCRIPTION

It is to be appreciated that certain aspects, modes, embodiments, variations and features of the present methods are described below in various levels of detail in order to provide a substantial understanding of the present technology.

In practicing the present methods, many conventional techniques in molecular biology, protein biochemistry, cell biology, immunology, microbiology and recombinant

DNA are used. *See, e.g.*, Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition; the series Ausubel *et al.* eds. (2007) *Current Protocols in Molecular Biology*; the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson *et al.* (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson *et al.* (1995) *PCR 2: A Practical Approach*; Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual*; Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition; Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Patent No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization*; Anderson (1999) *Nucleic Acid Hybridization*; Hames and Higgins eds. (1984) *Transcription and Translation; Immobilized Cells and Enzymes* (IRL Press (1986)); Perbal (1984) *A Practical Guide to Molecular Cloning*; Miller and Calos eds. (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); and Herzenberg *et al.* eds (1996) *Weir's Handbook of Experimental Immunology*. Methods to detect and measure levels of polypeptide gene expression products (*i.e.*, gene translation level) are well-known in the art and include the use of polypeptide detection methods such as antibody detection and quantification techniques. (*See also*, Strachan & Read, *Human Molecular Genetics*, Second Edition. (John Wiley and Sons, Inc., NY, 1999)).

The present disclosure provides a plasmid designated pAc/*emm*55 and methods for use in stimulating anticancer immunity in cancer patients.

In one embodiment, the vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) comprise plasmid DNA construct (pAc/emm55) formulated with in vivo-jetPEI®, a cationic polymer that aids in cellular uptake of DNA, and dextrose. The vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) can be administered by intralesional injection. When introduced into a patient's tumor cells, pAc/emm55 drives expression of the highly immunogenic Emm55 protein in the cytoplasm and on the surface of the tumor cells. The result is a clinically meaningful response against tumor cells, including tumor regression and prevention of recurrence.

No dose-limiting toxicities attributable to the vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) have been observed. IgG and IgM responses were seen in the peripheral blood to Emm55 peptides and known melanoma antigens, suggesting that the

vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) act as an individualized in-situ vaccine.

Combining the vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) with a targeted immune modulator, such as an anti-PD-1 antibody, can be used to enhance efficacy in treating cancer patients. The goal of combining these two therapies is to initiate a self-sustaining cycle of cancer immunity.

In some embodiments, the vaccines of the present technology can decrease the size of a tumor. In some embodiments, the vaccines of the present technology can decrease the rate of growth of the tumor.

In some embodiments, the vaccines of the present technology can decrease tumor heterogeneity, which refers to the existence of subpopulations of cells that can contain divergent biological behavior.

Definitions

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. For example, reference to "a cell" includes a combination of two or more cells, and the like. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, analytical chemistry and nucleic acid chemistry and hybridization described below are those well-known and commonly employed in the art.

As used herein, the term "about" in reference to a number is generally taken to include numbers that fall within a range of 1%, 5%, or 10% in either direction (greater than or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would be less than 0% or exceed 100% of a possible value).

As used herein, the "administration" of an agent or drug to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function. Administration can be carried out by any suitable route, including but not limited to, orally, intranasally, intrathecally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intrathecally, intraocularly, intradermally, transmucosally,

iontophoretically, or topically. Administration includes self-administration and the administration by another.

As used herein, the term “adaptive immune response” or “adaptive immune system response” refers to an antigen-mediated response by the immune system of a subject. A subject has a “deficient adaptive immune system response” when the subject’s adaptive immune system does not respond to one or more antigens as an active adaptive immune system would (*e.g.*, no T cell response or a diminished T cell response, or no B cell response or a diminished B cell response).

As used herein, the term “adjuvant” refers to a substance that enhances, augments, or potentiates the host’s immune response to antigens, including tumor antigens.

As used herein, the term “biological sample” means sample material derived from living cells. Biological samples may include tissues, cells, protein or membrane extracts of cells, and biological fluids (*e.g.*, ascites fluid or cerebrospinal fluid (CSF)) isolated from a subject, as well as tissues, cells and fluids present within a subject. Biological samples of the present technology include, but are not limited to, samples taken from breast tissue, renal tissue, the uterine cervix, the endometrium, the head or neck, the gallbladder, parotid tissue, the prostate, the brain, the pituitary gland, kidney tissue, muscle, the esophagus, the stomach, the small intestine, the colon, the liver, the spleen, the pancreas, thyroid tissue, heart tissue, lung tissue, the bladder, adipose tissue, lymph node tissue, the uterus, ovarian tissue, adrenal tissue, testis tissue, the tonsils, thymus, blood, hair, buccal, skin, serum, plasma, CSF, semen, prostate fluid, seminal fluid, urine, feces, sweat, saliva, sputum, mucus, bone marrow, lymph, and tears. Biological samples can also be obtained from biopsies of internal organs or from cancers. Biological samples can be obtained from subjects for diagnosis or research or can be obtained from non-diseased individuals, as controls or for basic research. Samples may be obtained by standard methods including, *e.g.*, venous puncture and surgical biopsy. In certain embodiments, the biological sample is a tissue sample obtained by needle biopsy.

As used herein, a "control" is an alternative sample used in an experiment for comparison purpose. A control can be "positive" or "negative." For example, where the purpose of the experiment is to determine a correlation of the efficacy of a therapeutic agent for the treatment for a particular type of disease, a positive control (a compound or

composition known to exhibit the desired therapeutic effect) and a negative control (a subject or a sample that does not receive the therapy or receives a placebo) are typically employed.

As used herein, a "cationic polymer" refers to a polymeric compound, linear or branched in nature, having one or more positive charges. Cationic polymers may be used to facilitate delivery of nucleic acids, which typically carry a negative charge, by forming a polymer-nucleic acid complex which can be taken up by cells at a greater rate than nucleic acids alone. Examples of cationic polymers include, but are not limited to, branched or linear, poly(ethyleneimine), poly(propylenimine), poly(amido amine), poly(β-amino ester), poly[2-(dimethylamino)ethyl methacrylate], and poly(L-lysine).

As used herein, the term "effective amount" or "therapeutically effective amount" refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, e.g., an amount which results in the prevention of, or a decrease in a disease or condition described herein or one or more signs or symptoms associated with a disease or condition described herein. In the context of therapeutic or prophylactic applications, the amount of a composition administered to the subject will vary depending on the composition, the degree, type, and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. The compositions can also be administered in combination with one or more additional therapeutic compounds. In the methods described herein, the therapeutic compositions may be administered to a subject having one or more signs or symptoms of a disease or condition described herein. As used herein, a "therapeutically effective amount" of a composition refers to composition levels in which the physiological effects of a disease or condition are ameliorated or eliminated. A therapeutically effective amount can be given in one or more administrations.

As used herein, "emm55" or "*emm55*" refers to the emm55 bacterial gene derived from *Streptococcus pyogenes* (*S. pyogenes*), or gene product produced therefrom (*e.g.*, emm55 protein). Emm55 is a serotyping protein normally expressed on the surface of the bacterium *S. pyogenes* and is highly antigenic but not rheumatogenic. In some embodiments, the *emm55* gene is encoded by a plasmid. In some embodiments, the *emm55* gene is encoded by a plasmid comprising SEQ ID NO: 1.

As used herein, "expression" includes one or more of the following: transcription of the gene into precursor mRNA; splicing and other processing of the precursor mRNA to produce mature mRNA; mRNA stability; translation of the mature mRNA into protein (including codon usage and tRNA availability); and glycosylation and/or other modifications of the translation product, if required for proper expression and function.

As used herein, the term "gene" means a segment of DNA that contains all the information for the regulated biosynthesis of an RNA product, including promoters, exons, introns, and other untranslated regions that control expression.

As used herein, the terms "increased", "increase" or "enhance" or "activate" are all used herein to generally mean an increase by a statically significant amount. For the avoidance of any doubt, the terms "increased", "increase" or "enhance" or "activate" means an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or greater as compared to a reference level.

As used herein, "immune checkpoint inhibitor" or "immune checkpoint blocking agent" or "immune checkpoint blockade agent" or "immune checkpoint blockade inhibitor" or "ICB inhibitor" refers to molecules that completely or partially reduce, inhibit, interfere with, or modulate the activity of one or more checkpoint proteins. Checkpoint proteins regulate T-cell activation or function. Checkpoint proteins include, but are not limited to, CD28 receptor family members, CTLA-4 and its ligands CD80 and CD86; PD-1 and its ligands PD-L1 and PD-L2; LAG3, B7-H3, B7-H4, TIM3, ICOS, II DLBCL, BTLA or any combination of two or more of the foregoing. Non-limiting examples of immune checkpoint blockade inhibitors contemplated for use herein include, but are not limited to, inhibitory antibodies against CD28 inhibitor such as CTLA-4 (cytotoxic T lymphocyte antigen 4) (*e.g.*, ipilimumab), anti-PD-1 (programmed Death 1) inhibitory antibodies (*e.g.*, nivolumab, pembrolizumab, pidilizumab, lambrolizumab), and anti-PD-L1 (Programmed death ligand 1) inhibitory antibodies (MPDL3280A, BMS-936559, MEDI4736, MSB

00107180), as well as inhibitory antibodies against LAG-3 (lymphocyte activation gene 3), TIM3 (T-cell immunoglobulin and mucin-3), B7-H3, TIGIT (T-cell immunoreceptor with Ig and ITIM domains), AMP-224, MDX-1105, arelumab, tremelimumab, IMP321, MGA271, BMS-986016, lirilumab, urelumab, PF-05082566, IPH2101, MEDI-6469, CP-870,893, Mogamulizumab, Varlilumab, Galiximab, AMP-514, AUNP 12, Indoximod, NLG-919, INCB024360, CD80, CD86, ICOS, DLBCL inhibitors, or BTLA, PDR001, and combinations thereof.

As used herein, the term "pharmaceutically-acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal compounds, isotonic and absorption delaying compounds, and the like, compatible with pharmaceutical administration. Pharmaceutically-acceptable carriers and their formulations are known to one skilled in the art and are described, for example, in Remington's Pharmaceutical Sciences (20th edition, ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, Pa.).

As used herein, the terms "polypeptide," "peptide" and "protein" are used interchangeably herein to mean a polymer comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.,* peptide isosteres. Polypeptide refers to both short chains, commonly referred to as peptides, glycopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. Polypeptides include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques that are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature.

As used herein, "prevention" or "preventing" of a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset of one or more symptoms of the disorder or condition relative to the untreated control sample.

As used herein, the terms "decrease", "reduced", "reduction", "decrease" or "inhibit" means a decrease by a statistically significant amount. For avoidance of doubt, "decrease", "reduced", "reduction", "decrease" or "inhibit" means a decrease by at least

10% as compared to a reference level, for example a decrease by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease (e.g., absent level as compared to a reference sample), or any decrease between 10-100% as compared to a reference level.

As used herein, the term "separate" therapeutic use refers to an administration of at least two active ingredients at the same time or at substantially the same time by different routes.

As used herein, the term "sequential" therapeutic use refers to administration of at least two active ingredients at different times, the administration route being identical or different. More particularly, sequential use refers to the whole administration of one of the active ingredients before administration of the other or others commences. It is thus possible to administer one of the active ingredients over several minutes, hours, or days before administering the other active ingredient or ingredients. There is no simultaneous treatment in this case.

As used herein, the term "simultaneous" therapeutic use refers to the administration of at least two active ingredients by the same route and at the same time or at substantially the same time.

As used herein, the terms "subject", "patient", or "individual" can be an individual organism, a vertebrate, a mammal, or a human. In some embodiments, the subject, patient, or individual is a human.

As used herein, the term "therapeutic agent" is intended to mean a compound that, when present in an effective amount, produces a desired therapeutic effect on a subject in need thereof.

"Treating" or "treatment" as used herein covers the treatment of a disease or disorder described herein, in a subject, such as a human, and includes: (i) inhibiting a disease or disorder, *i.e.*, arresting its development; (ii) relieving a disease or disorder, *i.e.*, causing regression of the disorder; (iii) slowing progression of the disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder. Therapeutic effects of treatment include, without limitation, inhibiting recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological

consequences of the disease, preventing metastases, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. By "treating a cancer" is meant that the symptoms associated with the cancer are, *e.g.*, alleviated, reduced, cured, or placed in a state of remission.

It is also to be appreciated that the various modes of treatment of disorders as described herein are intended to mean "substantial," which includes total but also less than total treatment, and wherein some biologically or medically relevant result is achieved. The treatment may be a continuous prolonged treatment for a chronic disease or a single, or few time administrations for the treatment of an acute condition.

The transitional term "comprising," which is synonymous with "including," or "containing," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. Use of the term "comprising" contemplates other embodiments that "consist" or "consist essentially of" the recited component(s).

In some particular embodiments of the present technology, the cancer treated is a melanoma. The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas include, for example, Harding-Passey melanoma, juvenile melanoma, lentigo melanoma, Cloudman's melanoma, S91 melanoma, nodular melanoma, subungal melanoma, and superficial spreading melanoma. In one embodiment, the cancer treated is skin cancer. Skin cancer includes, for example, melanoma and Merkel Cell Carcinoma.

The term "cytokine," as used herein, refers to a non-antibody protein that is released by one cell in response to contact with a specific stimulus. In some embodiments, the stimulus is an antigen. In some embodiments, the cytokine interacts with a second cell to mediate a response in the second cell. A cytokine can be endogenously expressed by a cell or administered to a subject. Cytokines may be released by immune cells, including macrophages, B cells, T cells, and mast cells to propagate an immune response. Cytokines can induce various responses in the recipient cell. Cytokines can include homeostatic cytokines, chemokines, pro-inflammatory cytokines, effectors, and acute-phase proteins.

For example, homeostatic cytokines, including interleukin (IL) 7 and IL-15, promote immune cell survival and proliferation, and pro-inflammatory cytokines can promote an inflammatory response. Examples of homeostatic cytokines include, but are not limited to, IL-2, IL-4, IL-5, IL-7, IL-10, IL-12p40, IL12p'70, IL-15, and interferon (IFN) gamma. Examples of pro-inflammatory cytokines include, but are not limited to, IL-1a, IL-1b, IL-6, IL-13, IL-17a, tumor necrosis factor (TNF)-alpha, TNF-beta, fibroblast growth factor (FGF) 2, granulocyte macrophage colony-stimulating factor (GM-CSF), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1), vascular endothelial growth factor (VEGF), VEGF-C, VEGF-D, and placental growth factor (PLGF). Examples of effectors include, but are not limited to, granzyme A, granzyme B, soluble Fas ligand (sFasK), and perforin. Examples of acute phase-proteins include, but are not limited to, C-reactive protein (CRP) and serum amyloid A (SAA).

Vaccine Compositions and Uses Thereof

In one aspect, the present disclosure provides an anti-cancer vaccine comprising a therapeutically effective amount of a nucleic acid sequence encoding *emm*55, a cationic polymer, and dextrose. In some embodiments the anti-cancer vaccine is a nucleic acid vaccine. In some embodiments, the nucleic acid vaccine is a DNA vaccine. In some embodiments, the nucleic acid sequence encoding *emm*55 comprises SEQ ID NO: 1, or a sequence having about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% similarity to SEQ ID NO: 1.

In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 0.1µg to about 100mg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 1µg to about 10,000µg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 1µg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 10µg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 20µg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 30µg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 40µg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 50µg. In some embodiments, the

therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 60μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 70μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 80μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 90μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 100μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 110μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 120μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 130μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 140μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 150μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 160μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 170μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 180μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 190μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 200μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 300μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 400μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 500μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 600μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 700μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 800μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 900μg. In some embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 1000μg. In some

embodiments, the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 100μg.

In some embodiments, the vaccine further comprises a pharmaceutically acceptable carrier. In some embodiments, the vaccine further comprises an adjuvant and/or an excipient. In some embodiments, the pharmaceutically acceptable carrier is selected from the group consisting of a cream, emulsion, gel, liposome, nanoparticle, or ointment. The skilled artisan would appreciate there are numerous pharmaceutically acceptable carriers that would be appropriate for use in conjunction with the vaccines of the present technology. In some embodiments, the vaccine is formulated for intralesional, pleural, topical, parenteral, intravenous, subcutaneous, intranodal, intratumoral, intrathecal, intrapleural or intraperitoneal administration. In some embodiments, the vaccine is formulated for intralesional administration. The skilled artisan will appreciate that formulation for a particular route of administration requires specific, physical attributes in the vaccine to be administered (*e.g.*, viscosity, concentration of active agent, etc.).

In some embodiments, the cationic polymer is linear or branched. In some embodiments, the cationic polymer is selected from the group consisting of poly(ethyleneimine), poly(propylenimine), poly(amido amine), poly(β-amino ester), poly[2-(dimethylamino)ethyl methacrylate], and poly(L-lysine). In some embodiments, the cationic polymer is a linear poly(ethyleneimine) or a branched poly(ethyleneimine). In some embodiments, the cationic polymer is a linear poly(ethyleneimine). In some embodiments, the vaccine comprises about 5μg to about 250mg cationic polymer. In some embodiments, the vaccine comprises about 50μg to about 250μg cationic polymer. In some embodiments, the vaccine comprises about 50μg cationic polymer. In some embodiments, the vaccine comprises about 60μg cationic polymer. In some embodiments, the vaccine comprises about 70μg cationic polymer. In some embodiments, the vaccine comprises about 80μg cationic polymer. In some embodiments, the vaccine comprises about 90μg cationic polymer. In some embodiments, the vaccine comprises about 100μg cationic polymer. In some embodiments, the vaccine comprises about 110μg cationic polymer. In some embodiments, the vaccine comprises about 120μg cationic polymer. In some embodiments, the vaccine comprises about 130μg cationic polymer. In some embodiments, the vaccine comprises about 140μg cationic polymer. In some embodiments, the vaccine

comprises about 144μg cationic polymer. In some embodiments, the vaccine comprises about 150μg cationic polymer. In some embodiments, the vaccine comprises about 160μg cationic polymer. In some embodiments, the vaccine comprises about 170μg cationic polymer. In some embodiments, the vaccine comprises about 180μg cationic polymer. In some embodiments, the vaccine comprises about 190μg cationic polymer. In some embodiments, the vaccine comprises about 200μg cationic polymer. In some embodiments, the vaccine comprises about 210μg cationic polymer. In some embodiments, the vaccine comprises about 220μg cationic polymer. In some embodiments, the vaccine comprises about 230μg cationic polymer. In some embodiments, the vaccine comprises about 240μg cationic polymer. In some embodiments, the vaccine comprises about 250μg cationic polymer.

In some embodiments, the vaccine comprises about 1% to about 20% dextrose. In some embodiments, the vaccine comprises about 1% dextrose. In some embodiments, the vaccine comprises about 2% dextrose. In some embodiments, the vaccine comprises about 3% dextrose. In some embodiments, the vaccine comprises about 4% dextrose. In some embodiments, the vaccine comprises about 5% dextrose. In some embodiments, the vaccine comprises about 6% dextrose. In some embodiments, the vaccine comprises about 7% dextrose. In some embodiments, the vaccine comprises about 8% dextrose. In some embodiments, the vaccine comprises about 9% dextrose. In some embodiments, the vaccine comprises about 10% dextrose. In some embodiments, the vaccine comprises about 11% dextrose. In some embodiments, the vaccine comprises about 12% dextrose. In some embodiments, the vaccine comprises about 13% dextrose. In some embodiments, the vaccine comprises about 14% dextrose. In some embodiments, the vaccine comprises about 15% dextrose. In some embodiments, the vaccine comprises about 16% dextrose. In some embodiments, the vaccine comprises about 17% dextrose. In some embodiments, the vaccine comprises about 18% dextrose. In some embodiments, the vaccine comprises about 19% dextrose. In some embodiments, the vaccine comprises about 20% dextrose.

In one aspect, the present disclosure provides a method for treating immune checkpoint inhibitor therapy resistant cancer in a subject in need thereof comprising administering to the subject the vaccine of the present technology, including any of the preceding embodiments. In some embodiments, the subject had previously been treated

with an immune checkpoint inhibitor therapy. In some embodiments, the subject had previously been treated with a PD-1 checkpoint inhibitor therapy. In some embodiments, the vaccine is administered intralesionally, pleurally, topically, parenterally, intravenously, subcutaneously, intranodally, intratumorally, intrathecally, intrapleurally, or intraperitoneally. In some embodiments, the vaccine is administered intralesionally.

In some embodiments, the method further comprises administering an additional therapeutic agent. In some embodiments, the additional therapeutic agent is administered simultaneously, sequentially, or separately to the vaccine. In some embodiments, the additional therapeutic agent is administered separately to the vaccine. In some embodiments, the additional therapeutic agent is administered after treatment with the vaccine.

In some embodiments, the additional therapeutic agent comprises an immune checkpoint inhibitor. In some embodiments, the immune checkpoint inhibitor comprises one or more of an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-TIM3 antibody, an anti-4-1BB antibody, an anti-CD73 antibody, an anti-GITR antibody, or an anti-LAG-3 antibody. In some embodiments, the immune checkpoint inhibitor comprises an anti-PD-1 antibody and/or an anti-PD-L1 antibody. In some embodiments, the anti-PD1 antibody is selected from the group consisting of Nivolumab, Pembrolizumab, Cemiplimab, Tislelizumab, Dostarlimab, Retifanlimab, and Toripalimab. In some embodiments, the anti-PD-L1 antibody is selected from the group consisting of Atezolizumab, Avelumab, Durvalumab, and Cosibelimab.

In some embodiments, the cancer is a solid tumor. In some embodiments, the tumor is a melanoma. In some embodiments, the melanoma is Merkel Cell Carcinoma.

In some embodiments, administration of the vaccine increases sensitivity of the cancer to treatment with checkpoint inhibitor therapy. In some embodiments, administration of the vaccine increases sensitivity of the cancer to treatment with PD1 checkpoint inhibitor therapy. In some embodiments, the PD-1 immune checkpoint inhibitor comprises an anti-PD-1 antibody and/or an anti-PD-L1 antibody. In some embodiments, the anti-PD1 antibody is selected from the group consisting of Nivolumab, Pembrolizumab, Cemiplimab, Tislelizumab, Dostarlimab, Retifanlimab, and Toripalimab. In some

embodiments, the anti-PD-L1 antibody is selected from the group consisting of Atezolizumab, Avelumab, Durvalumab, and Cosibelimab. In some embodiments, the PD1 checkpoint inhibitor therapy is selected from the group consisting of Nivolumab, Pembrolizumab, Cemiplimab, Tislelizumab, Dostarlimab, Retifanlimab, Toripalimab, Atezolizumab, Avelumab, Durvalumab, and Cosibelimab.

In some embodiments, administration of the vaccine increases expression of one or more genes selected from the group consisting of CXCL13, CD38, LAG3, STAT1, STAT2, CXCL11, CXCL13, IFI6, MX1, and LAG3. In some embodiments, administration of the vaccine decreases the expression of one or more genes selected from the group consisting of WNT2, WNT4, and PNOC.

The nucleotide sequence for pAc/*emm*55 is provided in **Table 1**.

Table 1: Nucleic Acid Sequence of pAc/emm55 Construct with Amino Acid Translation

Molecular Formula: plasmid DNA sequence with emm55 gene insert underlined, start and stop codons in **bold** and ***bold, italics*** font, respectively	TCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCATTAGT TATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTT CCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACC CCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGC AGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCT ACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTT TTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAA GTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGG GACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAG GCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAGA TCCGCTAGCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGT CGACGGTACCGCGGGCCCGGGATCCATAAGGAGCATAAAA**ATG**GCTAAAAATA CCACGAATAGACACTATTCGCTTAGAAAATTAAAAACAGGAACGGCTTCAGTA GCAGTAGCTTTGACTGTTTTAGGGACAGGACTGGTAGCAGGGCAGACAGTAAA AGCAAGCCAAACAGAACCATCTCAGACCAATAACAGATTATATCAAGAAAGAC AACGTTTACAGGATTTAAAAAGTAAGTTTCAAGACCTGAAAAATCGTTCAGAG GGATACATTCAGCAATACTACGACGAAGAAAAGAACAGTGGAAGTAACTCTAA CTGGTACGCAACCTACTTAAAAGAATTAAATGACGAATTTGAACAAGCTTATA ATGAACTTAGTGGTGATGGTGTAAAAAAATTAGCTGCAAGTTTGATGGAAGAA AGAGTCGCTTTAAGAGACGAAATCGATCAGATTAAGAAAATATCAGAAGAATT AAAAAATAAGCTGAGAGCAAAAGAAGAAGAATTAAAAAATAAAAAAGAGGAAC GTGAGCTTGAGCATGCTGCCTATGCAGCAGATGCAAAGAAACATGAAGAATAT GTCAAATCCATGTCTCTCGTACTAATGGATAAAGAAGAGGAGCGTCATAAACT AGAGCAATCATTAGACACGGCTAAAGCTGAGCTTGTTAAAAAAGAGCAAGAGT TACAGTTAGTCAAAGGCAATCTAGATCAAAAAGAAAAAGAACTAGAAAATGAA GAGCTAGCGAAAGAAAGTGCTATTAGTGATTTGACTGAGCAGATTACTGCTAA GAAGGCTGAAGTAGAAAAATTAACTCAAGATTTAGCTGCTAAGTCTGCTGAAA TTCAGGAAAAAGAAGCTGAAAAAGATCGCCAACAGCATATGTACGAAGCGTTT

	ATGAGCCAGTACAAAGAAAAAGTTGAGAAACAAGAGCAAGAGCTTGCTAAGCT AAAACAACTTGAAACCATCAACAACAATCTATTAGGTAATGCTAAGGATATGA TAGCTAAGTTGTCTGCTGAAAATGAACAATTAGCAAGCGACAAAGCAAAACTT GAAGAACAAAACAAGATTTCAGAAGCGAGCCGTAAAGGTCTTCGTCGTGACTT GGACGCATCACGTGAAGCTAAGAAACAAGTTGAAAAAGATTTAGCAAACTTGA CTGCTGAACTTGATAAGGTTAAAGAAGATAAACAAATTTCAGACGCAAGCCGT AAAGGTCTTCGTCGTGACTTGGACGCATCACGTGAAGCTAAGAAACAAGTTGA AAAAGCTTTAGAAGAAGCAAACAGCAAATTAGCGGCTCTTGAAAAACTTAACA AAGAGCTTGAAGAAAGCAAGAAATTAACAGAAAAAGAAAAAGCTGAGCTACAA GCGAAACTTGAAGCAGAAGCAAAAGCACTCAAAGAACAATTAGCGAAACAAGC TGAAGAACTTGCAAAACTAAGAGCTGGAAAAGCATCAGACTCACAAACCCCTG ATGCAAAACCAGGAAACAAAGTTGTTCCAGGTACAGGTCAAGCACCACAAGCA GGCACAAAACCTAACCAAAACAAAGCACCAATGAAGGAAACTAAGAGACAGTT ACCATCAACAGGTGAAGCAGCTAATCCATTCTTTACAGCGGCAGCCCTTACTG TTATGGCAACAGCTGGAGTAGCAGCAGTTGTAAAACGCAAAGAAGAAAACGAA GCTGAATTCTGCAGATATCCATCACACTGGCGGCCGCGACTC***TAG***ATCATAAT CAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACC TCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTT ATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAA TAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATG TATCTTAAGGCGTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAA TTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCC CTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGG AACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAAC CGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTT TGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGA TTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAA AGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCG TAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGC ACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACA TTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAAT ATTGAAAAAGGAAGAGTCCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTC AGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGC ATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGC AGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGC CCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCG CCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGC CTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTT GCAAAGATCGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGA TGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATG ACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCA GCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAA TGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTC CTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTA TTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGA GAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGG CTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACT CGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGG GCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGAGCATGCCCGACGGCG AGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAA AATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCG CTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCG AATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAG CGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGG

	TTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTC CACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCG GCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCT AGGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGC TATGACGGCAATAAAAAGACAGAATAAAACGCACGGTGTTGGGTCGTTTGTTC ATAAACGCGGGGTTCGGTCCCAGGGCTGGCACTCTGTCGATACCCCACCGAGA CCCCATTGGGGCCAATACGCCCGCGTTTCTTCCTTTTCCCCACCCCACCCCCC AAGTTCGGGTGAAGGCCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCTG CCATAGCCTCAGGTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTT TAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAAT CCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCA AAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACA AAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAAC TCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTC TTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCT ACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAA GTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGC GGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACC TACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCC CGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAG AGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTC GGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGG GCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCT TTTGCTGGCCTTTTGCTCACATGTTCTT (**SEQ ID NO: 1**)
Amino acid translation of emm55 gene sequence (566 AA)	MAKNTTNRHYSLRKLKTGTASVAVALTVLGTGLVAGQTVKASQTEPSQTNNRL YQERQRLQDLKSKFQDLKNRSEGYIQQYYDEEKNSGSNSNWYATYLKELNDEF EQAYNELSGDGVKKLAASLMEERVALRDEIDQIKKISEELKNKLRAKEEELKN KKEERELEHAAYAADAKKHEEYVKSMSLVLMDKEEERHKLEQSLDTAKAELVK KEQELQLVKGNLDQKEKELENEELAKESAISDLTEQITAKKAEVEKLTQDLAA KSAEIQEKEAEKDRQQHMYEAFMSQYKEKVEKQEQELAKLKQLETINNNLLGN AKDMIAKLSAENEQLASDKAKLEEQNKISEASRKGLRRDLDASREAKKQVEKD LANLTAELDKVKEDKQISDASRKGLRRDLDASREAKKQVEKALEEANSKLAAL EKLNKELEESKKLTEKEKAELQAKLEAEAKALKEQLAKQAEELAKLRAGKASD SQTPDAKPGNKVVPGTGQAPQAGTKPNQNKAPMKETKRQLPSTGEAANPFFTA AALTVMATAGVAAVVKRKEENEAEFCRYPSHWRPRL (**SEQ ID NO: 2**)

The vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) were shown to be safe to inject into melanoma lesions, and there were no dose limiting toxicities attributable to them. The vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) appear to act through the interferon response and Toll-Like Receptors. There are IgG and IgM antigen-specific responses to both the Emm55 protein and melanoma-specific antigens in patient-derived plasma. Post-treatment evidence of clinical benefit for retreatment with anti-PD1 antibodies in three patients previously refractory to those agents is encouraging and suggests synergy when the vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) are administered in conjunction with immunotherapy.

When the vaccine is administered in vivo into a solid cancer, a polypeptide expressed by the vaccine enhances and/or augments an immune response to the targeted tumor.

With the vaccine, injections can be administered directly into tumor lesions using lipid reagents, needless injectors, multi-needle administration patches, in vivo electroporation, J-tip, into palpable tissue, or visceral tumor lesions with the guidance of computed tomography (CT) or ultrasound. Advantageously, in using the direct administration of the vaccine, there is no need to harvest tumor cells from the patient. In one embodiment, tumor cells are not harvested.

In one embodiment, the vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) comprise a plasmid DNA construct (pAc/emm55) formulated with in vivo-jetPEI®, a cationic polymer that aids in cellular uptake of DNA, and dextrose. The vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) can be administered by intralesional injection. When introduced into a patient's tumor cells, pAc/emm55 drives expression of the highly immunogenic Emm55 protein in the cytoplasm and on the surface of the tumor cells. The result is a clinically meaningful response against tumor cells, including tumor regression and prevention of recurrence.

No dose-limiting toxicities attributable to the vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) have been observed. IgG and IgM responses were seen in the peripheral blood to Emm55 peptides and known melanoma antigens, suggesting that the vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) act as an individualized in-situ vaccine.

Combining the vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) with a targeted immune modulator, such as an anti-PD-1 antibody, can be used to enhance efficacy in treating cancer patients. The goal of combining these two therapies is to initiate a self-sustaining cycle of cancer immunity.

The vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) can be provided as a kit containing one vial of plasmid DNA (pAc/emm55 in TE buffer) and one vial of cationic polymer (in vivo-jetPEI® (linear polyethyleneimine)). Glucose (10% dextrose solution for injection). The vaccine composition components (*e.g.* plasmid DNA, cationic polymer, and glucose) can be combined at the clinical site in preparation for intralesional injection of the vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) on a per-patient basis. Vaccine kits can be transported to the clinical site in a 2-8 °C pre-validated temperature-controlled shipping container with a temperature monitor.

One embodiment of the vaccine compositions of the present technology (*e.g.*, IFx-Hu2.0) for clinical use is described in Table 2. Each dose contains 100 μg of plasmid DNA at a concentration of 0.5 μg/μL in a total volume of 200 μL.

Table 2: Final IFx-Hu2.0 Drug Product Components

Component	Description	Purpose
Plasmid DNA	Mammalian expression plasmid containing emm55 gene (pAc/emm55) in TE buffer (0.5 mg/mL)	Drug substance
Cationic Polymer	*Polyplus-transfection in vivo-jetPEI® cationic polymer (0.72 mg/mL)*	Excipient; cellular uptake of plasmid DNA
Glucose, 10% Dextrose solution for injection, USP[1]	Dextrose (50 mg/mL)	Excipient; DNA-polymer complex stability

[1]Glucose (10% Dextrose Solution for Injection, USP) will be sourced directly by the clinical site and not provided by Sponsor in the kit. The final concentration of dextrose compounded at the clinical site is 50 mg/mL or 5%.

Immune Checkpoint Inhibitors

An important part of the immune system is its ability to keep itself from attacking normal cells in the body. To do this, it uses "checkpoints", which are proteins on immune cells that need to be turned on (or off) to start an immune response. Melanoma cells sometimes use these checkpoints to avoid being attacked by the immune system. Checkpoint inhibitors target the checkpoint proteins, helping to restore the immune response against melanoma cells.

Keytruda®(pembrolizumab) is a drug that targets PD-1, a protein on immune system cells called T cells that normally help keep these cells from attacking other cells in the body. By blocking PD-1, these drugs boost the immune response against melanoma cells.

These drugs can be given as an intravenous (IV) infusion every 2 or 3 weeks. Side effects of these drugs can include fatigue, cough, nausea, itching, skin rash, decreased appetite, constipation, joint pain, and diarrhea. Other, more serious side effects occur less often. Sometimes the immune system starts attacking other parts of the body, which can cause serious or even life-threatening problems in the lungs, intestines, liver, hormone-making glands, kidneys, or other organs.

In some embodiments, the vaccines of the present technology can be used in conjunction with monoclonal antibodies and/or checkpoint inhibitory molecules such as CTLA-4, PD-1, PD-L1, PD-L2, LAG3, TIM3, TIGIT. Antibodies can be, for example, costimulatory molecules such as CD40, OX40; antibodies capable of regulating T regs such as anti-GITR and pan anti-BCL-2; or cytokines such as IL-2, TNF-α, IFN-γ, IFN-β, and TLR agonists. The *emm55* vector can also be used with additional nucleic acids that expresses immunologic molecules such as cytokines IL-2, IL-12, IL-18, and MHC genes. These will augment anti-tumor immunity.

Decreasing Resistance to Cancer Treatments

In one aspect, the present disclosure describes methods for sensitizing immune checkpoint therapy resistant cancers to treatment. Some cancers, whether previously treated with checkpoint inhibitor therapies or not, are resistant to immune checkpoint therapy. However as shown in the Experimental Examples, the vaccines and methods of the present disclosure can re-sensitize checkpoint inhibitor therapy resistant tumors, resulting in improved treatment outcomes for subjects.

Resistance to therapy in cancer can be affected by tumor burden and growth kinetics, tumor heterogeneity, physical barriers, the immune system and the microenvironment, undruggable cancer drivers, and any combination thereof. The vaccines of the present technology can address these determinants of drug resistance.

In some embodiments, the vaccines of the present technology can address physical barriers of cancer treatments by injecting the compositions directly into a tumor. The *emm55* DNA vector expressing an immunogenic polypeptide can be injected into a solid cancer. The DNA vector enters the tumor cells in vivo and expresses a highly immunogenic polypeptide. The polypeptide stimulates an immune response in vivo to that cancer, acting as an internally generated immuno-stimulant. Use of therapeutic DNA vectors avoids having to isolate, culture and transform tumor cells in vitro because the vectors can be introduced directly into a solid tumor mass.

Formulations

The compositions of the present technology can be manufactured by methods well known in the art such as conventional granulating, mixing, dissolving, encapsulating, lyophilizing, or emulsifying processes, among others. Compositions may be produced in various forms, including granules, precipitates, or particulates, powders, including freeze dried, rotary dried or spray dried powders, amorphous powders, tablets, capsules, syrup,

suppositories, injections, emulsions, elixirs, suspensions or solutions. Formulations may optionally contain solvents, diluents, and other liquid vehicles, dispersion or suspension aids, surface active agents, pH modifiers, isotonic agents, thickening or emulsifying agents, stabilizers and preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. In certain embodiments, the compositions disclosed herein are formulated for administration to a mammal, such as a human.

Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, cyclodextrins, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables. The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use. Compositions formulated for

parenteral administration may be injected by bolus injection or by timed push, or may be administered by continuous infusion.

In order to prolong the effect of a compound of the present disclosure, it is often desirable to slow the absorption of the compound from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the compound then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered compound form is accomplished by dissolving or suspending the compound in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the compound in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of compound to polymer and the nature of the particular polymer employed, the rate of compound release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the compound in liposomes or microemulsions that are compatible with body tissues.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents such as phosphates or carbonates.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

Experimental Examples

The present technology is further illustrated by the following Examples, which should not be construed as limiting in any way.

Example 1: *In vivo* Efficacy of the Vaccines of the Present Disclosure

A. Materials and Methods

To test safety, tolerability, and feasibility, IFx-Hu2.0, see **Table 1** for plasmid sequence, was injected into cutaneous lesions of adult melanoma patients (≥18 years) with unresectable stage III/IV cutaneous melanoma. An exemplary dose of IFx-Hu2.0 is shown in **Table 2.** At least two injectable lesions ≥3 mm in size were required for entry into the study. Subjects were all evaluated for anti-PD-1 therapy, TVEC, and/or BRAF inhibition as indicated in the FDA labels for those agents and were deemed not candidates, defined as refractory to approved therapies or unable (due to immune-based diseases)/unwilling to

tolerate the adverse effects of these agents. Small brain metastases (≤1 cm) were permitted, assuming a longer than 3-month life expectancy and any received concurrent radiation was at a site distant from intralesional injection. Subjects could not receive any other concurrent anticancer therapies, nor could they have uncontrolled hepatitis/HIV infection, organ transplantation, or immunosuppression requiring more than 10 mg prednisone-equivalent daily. Trial subjects had the option of continued dosing every three weeks if they tolerated the first dose and showed no evidence of progression. The study's success was defined as treating 5/6 subjects without dose-limiting toxicity (DLT) at 28 days.

Biopsies were taken before and after treatment, snap frozen, and/or placed in formalin for FFPE embedding. In addition, plasma samples were collected before the first dose and during the four-week DLT period. Plasma was separated from peripheral blood samples as previously described[16].

Frozen tissue analysis: mRNA was extracted from frozen tissue specimens using the RNAeasy UCP Micro kit and shipped to Nanostring® for analysis using the PanCancer IO360 Expression Panel[17]. At the same time points of tissue collection, paired plasma samples were collected.

Plasma analyses: Plasma samples were screened on Olink Inflammation (INF, v.3022) and Immunooncology (IO, v.3111) panels to measure cytokines and chemokines in plasma as per manufacturer instructions[18, 19]. Plasma samples were also subjected to melanoma-associated antigen-antibody IgG and IgM response profiling. Pre- and post-treatment plasma samples from subjects were diluted 1:100. Samples from subject M10106 were diluted 1:250. Diluted samples were screened on PEPperPRINT PEPperCHIP® Melanoma Antigen Microarray for 21 melanoma antigens as per manufacturer instructions[18, 19]. Hemagglutinin (HA), GS linker and polio control peptides were included on the chip as a series of 15 amino acid peptides. Subject antibody binding was detected using goat anti-human IgG (Fc) DyLight680 (0.1 μg/ml) and goat anti-human IgM (μ chain) DyLight800 (0.2 μg/ml) and imaged on a LI-COR Odyssey Imaging System. In addition, the PEPperPRINT technology was utilized to analyze the Emm55-specific IgG and IgM antibody responses. The Emm55 amino acid sequence was converted into 15 amino acid peptides with an overlap of 14 amino acids. The neutral linkers were printed in duplicate on a custom PEPperPRINT PEPperCHIP®, which also contained HA and polio controls. Pre- and post-treatment plasma samples were screened at a dilution of 1:150. Staining was

identical to above except an Innopsys InnoScan 710-IR Microarray Scanner was used to image the slides.

Statistical Analyses

Differential expression analysis of Nanostring mRNA data was analyzed using the DESeq2 method[20]. A volcano plot was constructed to depict a log2 fold change of mRNA expression levels measured in the patient's tissue pre- vs. post therapy and a p-value derived from DESeq2 method. Significant genes (log2-fold, p-value <0.05) were analyzed via core analysis in Ingenuity® pathway analysis and visualized within the graphical summary tab. Box plots were also constructed to analyze trends for individual mRNA in IFx-Hu2.0-injected vs. uninjected lesions. To identify differentially abundant proteins between pre- and post-treatment samples for each patient, an outlier linear regression analysis test was performed for each Olink INF and IO panel data[21]. In brief, studentized residuals, i.e., the scaled distance between the observed value and predicted value, were calculated, and proteins with absolute studentized residuals of >1.97 were identified to have differential abundance. Similar analyses were performed to measure IgG and IgM on the PEPperPRINT melanoma chip. In addition, the appearance or disappearance of recognition of melanoma antigens in plasma was depicted via a heat map graph.

B. Results

Clinical results

Eight subjects were screened (M10101-8). One subject failed screening (M10102), while seven met eligibility requirements for the trial. Subjects ranged in age from 60 to 85. The primary endpoint was met in that six patients who completed the dose-limiting toxicity period experienced no DLT related to IFx-Hu2.0. Only grade 1 and 2 toxicities deemed related to IFx-Hu2.0 were encountered. These were expected for intralesional injection (i.e., injection site reactions) and managed with conservative measures. In brief: observed toxicities included: grade 1-2 injection site reactions in 5 of 7 patients; grade 1 bleeding - 1/7; grade 1-2 Pain - 2/7, grade 1 lymphopenia - 1/7, grade 1 pruritis - 1/7. No grade 3 or greater toxicities related to study drug were observed. One grade 5 toxicity (death due to *Clostridium septicum* infection 20 days post injection at a site distant from study drug injection sites) was deemed unlikely related to the study drug after a thorough investigation, including autopsy illustrating likely left groin source of infection. Six patients had injections at one time point and one patient had injections at two time points. Three patients had stable disease and three patients progressed by RECIST 1.1 at the 30-day follow-up visit. LDH

values are within normal limits for all evaluable patients pre/post therapy and there are no significant differences in neutrophil/lymphocyte ratios.

Post-protocol therapy responses

Of the 7 patients, 6 received further anticancer treatment after coming off protocol. Surprisingly, 3 of 4 anti-PD-1 refractory patients had evidence of clinical benefit after post-protocol retreatment with anti-PD1 based therapy (stable disease (SD) lasting >two years followed by surgical resection with PFS post-protocol therapy (PFS2) >3.66 years; PFS2 of 1.08 years; and PR subsequently surgical resected and rendered no evidence of disease - PFS2 of 1.66 years). PFS2 on subsequent anti-PD-1 therapy is depicted in and demonstrates increased PFS2 compared to historical controls post-anti-PD-1 failure.

Effects of IFx-Hu2.0 on promoting immune responses

Paired samples from each patient collected pre- and approximately 30 days post-injection were analyzed via the mRNA panel. Multiple genes associated with B cell antibody-dependent immune responses, including CXCL13, and CD38, were upregulated ($p < 0.05$) in lesions biopsied post-therapy on protocol, in addition to immune checkpoint molecules such as LAG3 and proteins indicative of a strong interferon response (STAT1, STAT2, and multiple IRFs). Both innate and adaptive immune responses were detected in the injected lesions. Genes associated with anti-PD1 or LAG3 response, such as CXCL11, CXCL13, IFI6, MX1, and LAG3 were upregulated ($p<0.05$). In addition, several oncogenes were downregulated (WNT2, WNT4 and PNOC). A strong interferon response was noted along with processes involved in antigen presentation and anti-viral responses.

Cytokines were measured, as well as melanoma and Emm55-specific peptide antigens in the plasma. Cytokine/chemokine analysis demonstrated a variable response in patient-derived plasma samples, suggesting that an individualized immune response is generated by IFx-Hu2.0 activity. Although all patients had increased antibody production against melanoma-specific peptides, the combination of peptides recognized by each patient was unique. In each patient, IgG and IgM reactivity to overlapping Emm55 peptides (5-14 amino acid residues) was also detected but the peptides recognized were unique for each patient. The antibody responses to Emm55-specific and melanoma-specific peptides increased after therapy. In many cases, antibody responses were present after treatment where none existed before therapy. IgG and IgM responses to individual peptides differ for different patients.

These results demonstrate that the methods and compositions of the present technology are effective for treating cancer (*e.g.*, melanoma cancer) and for resensitizing tumors to treatment with checkpoint blockade inhibitors (*e.g.*, anti PD1 based therapies). Accordingly, the methods and compositions of the present technology are useful for the treatment of checkpoint inhibitor resistant tumors.

EQUIVALENTS

The present technology is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of this present technology can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the present technology, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the present technology. It is to be understood that this present technology is not limited to particular methods, reagents, compounds compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like, include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

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WHAT IS CLAIMED IS

1. An anti-cancer vaccine comprising a therapeutically effective amount of a nucleic acid sequence encoding *emm*55, a cationic polymer, and dextrose.

2. The vaccine of claim 1, wherein the nucleic acid sequence encoding *emm*55 comprises SEQ ID NO: 1.

3. The vaccine of claims 1 or 2, wherein the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 1μg to about 10,000μg.

4. The vaccine of any one of claims 1-3, wherein the therapeutically effective amount of the nucleic acid sequence encoding *emm55* is about 100μg.

5. The vaccine of any one of claims 1-4, wherein the vaccine further comprises a pharmaceutically acceptable carrier selected from the group consisting of a cream, emulsion, gel, liposome, nanoparticle, or ointment.

6. The vaccine of any one of claims 1-5, wherein the vaccine is formulated for intralesional, pleural, topical, parenteral, intravenous, subcutaneous, intranodal, intratumoral, intrathecal, intrapleural or intraperitoneal administration.

7. The vaccine of any one of claims 1-6, wherein the vaccine is formulated for intralesional administration.

8. The vaccine of any one of claims 1-7, wherein the cationic polymer is selected from the group consisting of poly(ethyleneimine), poly(propylenimine), poly(amido amine), poly(β-amino ester), poly[2-(dimethylamino)ethyl methacrylate], and poly(L-lysine).

9. The vaccine of any one of claims 1-8, wherein the cationic polymer is a linear poly(ethyleneimine) or a branched poly(ethyleneimine).

10. The vaccine of any one of claims 1-9, wherein the vaccine comprises about 50μg to about 250μg cationic polymer.

11. The vaccine of any one of claims 1-10, wherein the vaccine comprises about 144μg cationic polymer.

12. The vaccine of any one of claims 1-11, wherein the vaccine comprises about 1% to about 20% dextrose.

13. The vaccine of any one of claims 1-12, wherein the vaccine comprises about 10% dextrose.

14. A method for treating immune checkpoint inhibitor therapy resistant cancer in a subject in need thereof comprising administering to the subject the vaccine of any one of claims 1-13.

15. The method of claim 14, wherein the subject had previously been treated with a PD-1 checkpoint inhibitor therapy.

16. The method of claims 14 or 15, wherein the vaccine is administered intralesionally, pleurally, topically, parenterally, intravenously, subcutaneously, intranodally, intratumorally, intrathecally, intrapleurally, or intraperitoneally.

17. The method of claim 16, wherein the vaccine is administered intralesionally.

18. The method of any one of claims 14-17 further comprising administering an additional therapeutic agent.

19. The method of claim 18, wherein the additional therapeutic agent is administered simultaneously, sequentially, or separately to the vaccine.

20. The method of claim 18 or claim 19, wherein the additional therapeutic agent comprises an immune checkpoint inhibitor.

21. The method of claim 20, wherein the immune checkpoint inhibitor comprises one or more of an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-TIM3 antibody, an anti-4-1BB antibody, an anti-CD73 antibody, an anti-GITR antibody, or an anti-LAG-3 antibody.

22. The method of claim 20 or 21, wherein the immune checkpoint inhibitor comprises an anti-PD-1 antibody and/or an anti-PD-L1 antibody.

23. The method of claims 21 or 22, wherein the anti-PD1 antibody is selected from the group consisting of Nivolumab, Pembrolizumab, Cemiplimab, Tislelizumab, Dostarlimab, Retifanlimab, and Toripalimab.

24. The method of any one of claims 21-22, wherein the anti-PD-L1 antibody is selected from the group consisting of Atezolizumab, Avelumab, Durvalumab, and Cosibelimab.

25. The method of any one of claims 14-24, wherein the cancer is a solid tumor.

26. The method of claim 25, wherein the tumor is a melanoma.

27. The method of claim 26, wherein the melanoma is Merkel Cell Carcinoma.

28. The method of any one of claims 14-27, wherein administration of the vaccine increases sensitivity of the cancer to treatment with PD1 checkpoint inhibitor therapy.

29. The method of claim 28, wherein the PD1 checkpoint inhibitor therapy is selected from the group consisting of Nivolumab, Pembrolizumab, Cemiplimab, Tislelizumab, Dostarlimab, Retifanlimab, Toripalimab, Atezolizumab, Avelumab, Durvalumab, and Cosibelimab.

30. The method of any one of claims 14-29, wherein administration of the vaccine increases expression of one or more genes selected from the group consisting of CXCL13, CD38, LAG3, STAT1, STAT2, CXCL11, CXCL13, IFI6, MX1, and LAG3.

31. The method of any one of claims 14-30, wherein administration of the vaccine decreases the expression of one or more genes selected from the group consisting of WNT2, WNT4, and PNOC.