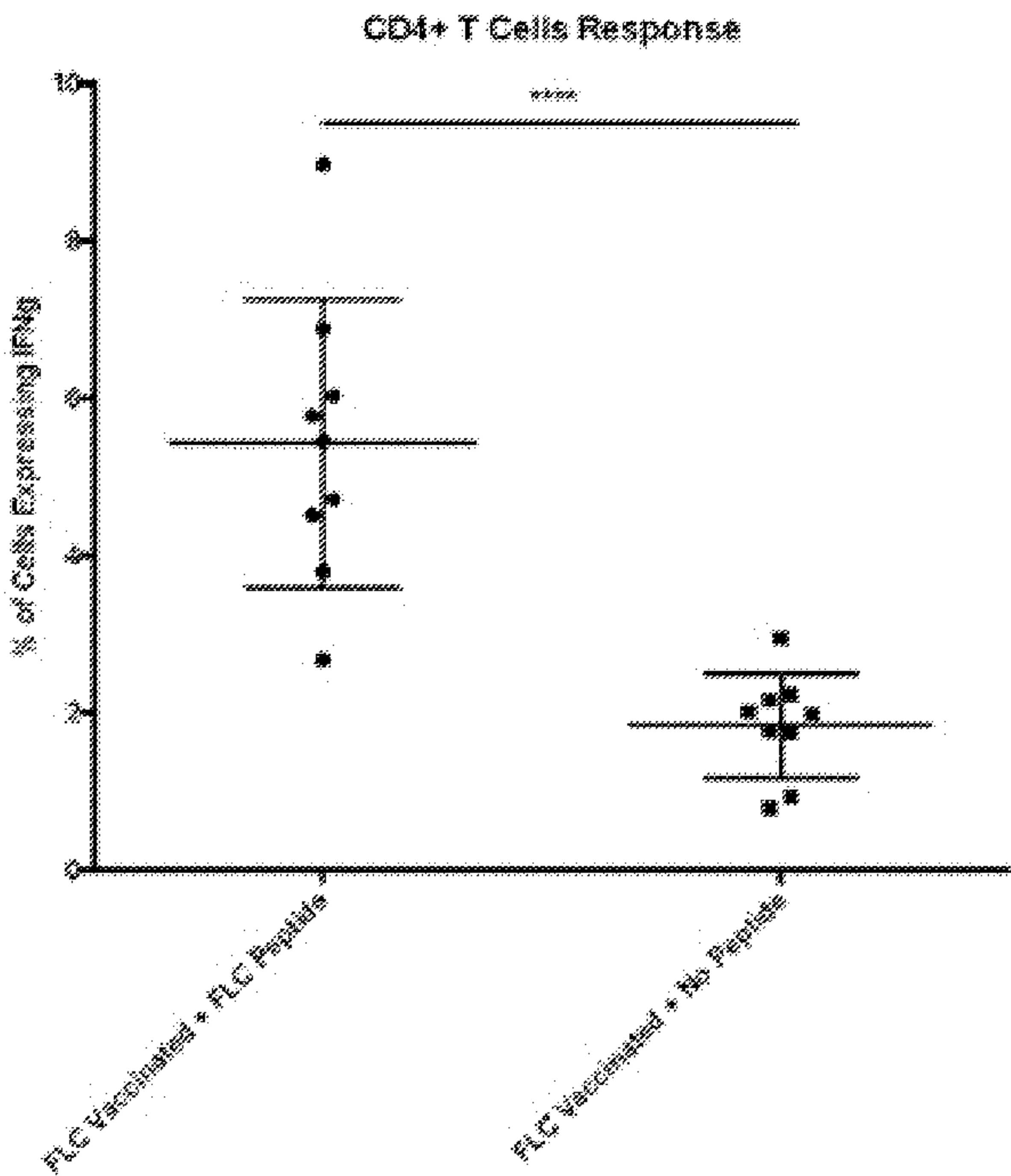




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- (71) **Applicant: THE JOHNS HOPKINS UNIVERSITY**
[US/US]; 3400 N Charles Street, Baltimore, Maryland 21218 (US).
- (72) **Inventors: YARCHOAN, Mark**; 1812 Ashland Avenue, Suite 110, Baltimore, Maryland 21205 (US). **JAFFEE, Elizabeth A.**; 20 Summer Fields Court, Lutherville, Maryland 21093 (US). **MOHAN, Aditya**; 1812 Ashland Avenue, Suite 110, Baltimore, Maryland 21205 (US).
- (74) **Agent: CORLESS, Peter F.**; Mintz Levin, One Financial Center, Boston, Massachusetts 02111 (US).
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(54) **Title:** FUSION PROTEINS AND METHODS OF USE THEREOF

FIG. 1



(57) **Abstract:** The invention features compositions and methods for treating and preventing cancer. In one aspect, isolated fusion proteins are provided that comprise a DNAJB1 portion and a PRKACA portion. In a further aspect, compositions are provided, including immunogenic compositions that comprise an isolated fusion protein comprising a DNAJB1 portion and a PRKACA portion. In a yet further aspect, a cancer vaccine is provided that comprises an isolated fusion protein comprising a DNAJB1 portion and a PRKACA portion.

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FUSION PROTEINS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Application No. 62/955,641, filed December 31, 2019, the entire contents of which is incorporated herein by reference in its entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

The contents of the sequence listing text file named “48317-579001WO_Sequence_Listing_ST25.txt”, which was created on December 29, 2020 and is 16,384 bytes in size, is hereby incorporated by reference in its entirety.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was made with government support under Grant Number P50 CA062924, awarded by the National Cancer Institute, and under Grant Number P30 CA006973, awarded by the National Institutes of Health. The Government has certain rights in the invention.

FIELD

This invention relates generally to the field of oncology.

BACKGROUND

Fibrolamellar hepatocellular carcinoma (FLC) is a rare and often lethal form of liver cancer that typically affects adolescents and young adults without underlying cirrhosis. There is no standardized systemic therapy for this cancer, and patients with unresectable disease have a median survival of only 12 months. As such, there is a pressing need to identify additional treatment options for PDA.

SUMMARY

The invention is based, at least in part, on the surprising discovery of fusion peptides that facilitate effector T cell immune response and can be used as cancer vaccines to treat or prevent cancer. Pediatric liver cancer (e.g., Fibrolamellar Hepatocellular Carcinoma (FLC)) expresses a fusion protein, which joins the J domain of heat shock protein genes (e.g., DNAJB1) to the kinase domain of a cAMP-dependent protein kinase (e.g., PRKACA). A

corresponding fusion protein comprising a DNAJB1 portion and a PRKACA portion is capable of inducing T cell immune response for a cancer vaccine therapy to treat or prevent these cancers.

In one aspect, isolated fusion proteins are provided that comprise a DNAJB1 portion and a PRKACA portion.

In a further aspect, compositions are provided, including immunogenic compositions that comprise an isolated fusion protein comprising a DNAJB1 portion and a PRKACA portion.

In a yet further aspect, a cancer vaccine is provided that comprises an isolated fusion protein comprising a DNAJB1 portion and a PRKACA portion.

In some embodiments, the DNAJB1 portion comprises at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more, sequence identity to a DNAJB1 protein. In some embodiments, the DNAJB1 portion comprises at least 70% sequence identity to SEQ ID NO: 1, 2, 5, or 7. In some embodiments, the DNAJB1 portion comprises at least 90% sequence identity to SEQ ID NO: 1, 2, 5, or 7. In some embodiments, the DNAJB1 portion comprises SEQ ID NO: 1, 2, 5, or 7.

In some embodiments, the PRKACA portion comprises at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or more, sequence identity to a PRKACA protein. In some embodiments, the PRKACA portion comprises at least 70%, 80% or 85% sequence identity to SEQ ID NO: 3, 4, 6, or 8. In some embodiments, the PRKACA portion comprises at least 90% sequence identity to SEQ ID NO: 3, 4, 6, or 8. In some embodiments, the PRKACA portion comprises SEQ ID NO: 3, 4, 6, or 8.

In some embodiments, the DNAJB1 portion is fused to the N-terminus of the PRKACA portion. In some embodiments, the DNAJB1 portion is fused to the C-terminus of the PRKACA portion.

In some embodiments, the DNAJB1 portion and the PRKACA portion are directly fused. In some embodiments, the DNAJB1 portion and the PRKACA portion are fused through a linker. Such linker may be any chemical linker or peptide linker known to a skilled artisan.

In some embodiments, the fusion protein, composition and/or cancer vaccine described herein comprises at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or more, sequence identity to a DNAJB1-PRKACA fusion protein. In some embodiments, the fusion protein, composition and/or cancer vaccine comprises at least 70%, 80% or 85% sequence identity to SEQ ID NO: 9. In some embodiments, the fusion

protein, composition and/or cancer vaccine comprises at least 90% sequence identity to SEQ ID NO: 9. In some embodiments, the cancer vaccine comprises SEQ ID NO: 9.

In some embodiments, the fusion protein, composition and/or cancer vaccine described herein induces immune response in a subject expressing a DNAJB1-PRKACA fusion protein.

In some embodiments, the subject has Fibrolamellar hepatocellular carcinoma (FLC), pancreatic cancer, biliary cancer, lung cancer, or another cancer that contains the DNAJB1-PRKACA fusion protein.

In some embodiments, the fusion protein, composition and/or cancer vaccine further comprises an adjuvant.

In some embodiments, the composition or cancer vaccine further comprises an immune checkpoint inhibitor, or an immune checkpoint inhibitor is administered together, in combination or otherwise in conjunction with the composition or cancer vaccine. Such immune checkpoint inhibitor may include, at least, antibodies or other inhibiting agents to checkpoint inhibitors targeting program cell death protein 1 (PD-1), program cell death-ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), or combinations thereof.

In some embodiments, the fusion protein, composition and/or cancer vaccine induces CD4 and/or CD8 T cell response.

Another aspect of the invention provides for a method of treating or preventing cancer in a subject comprising:

administering a fusion protein, composition or vaccine as disclosed herein to the subject;

thereby treating or preventing said cancer in said subject, wherein the fusion protein, composition or vaccine comprises an isolated fusion protein comprising a DNAJB1 portion and a PRKACA portion.

In some embodiments, the DNAJB1 portion comprises at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99%, or more, sequence identity to a DNAJB1 protein. In some embodiments, the DNAJB1 portion comprises at least 70% sequence identity to SEQ ID NO: 1, 2, 5, or 7. In some embodiments, the DNAJB1 portion comprises at least 90% sequence identity to SEQ ID NO: 1, 2, 5, or 7. In some embodiments, the DNAJB1 portion comprises SEQ ID NO: 1, 2, 5, or 7.

In some embodiments, the PRKACA portion comprises at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or more, sequence identity to

a PRKACA protein. In some embodiments, the PRKACA portion comprises at least 70% sequence identity to SEQ ID NO: 3, 4, 6, or 8. In some embodiments, the PRKACA portion comprises at least 90% sequence identity to SEQ ID NO: 3, 4, 6, or 8. In some embodiments, the PRKACA portion comprises SEQ ID NO: 3, 4, 6, or 8.

In some embodiments, the fusion protein comprises SEQ ID NO: 9.

In some embodiments, the DNAJB1 portion comprises SEQ ID NO: 1 fused to a PRKACA portion comprising SEQ ID NOS: 3, 4, 6, or 8.

In some embodiments, the DNAJB1 portion comprises SEQ ID NO: 2 fused to a PRKACA portion comprising SEQ ID NOS: 3, 4, 6, or 8.

In some embodiments, the DNAJB1 portion comprises SEQ ID NO: 5 fused to a PRKACA portion comprising SEQ ID NOS: 3, 4, 6, or 8.

In some embodiments, the DNAJB1 portion comprises SEQ ID NO: 7 fused to a PRKACA portion comprising SEQ ID NOS: 3, 4, 6, or 8.

In some embodiments, one or more DNAJB1 sequences are fused to two or more PRKACA sequences.

In some embodiments, two or more DNAJB1 sequences are fused to two or more PRKACA sequences.

In some embodiments, two or more DNAJB1 sequences are fused to two or more PRKACA sequences. In some embodiments, the fusion protein may comprise tandem repeats of a DNAJB1 portion fused to a PRKACA portion.

In some embodiments, the DNAJB1 portion is fused to the N-terminus of the PRKACA portion. In some embodiments, the DNAJB1 portion is fused to the C-terminus of the PRKACA portion.

In some embodiments, the DNAJB1 portion and the PRKACA portion are directly fused. In some embodiments, the DNAJB1 portion and the PRKACA portion are fused through a linker. Such linker may be any chemical linker or peptide linker known to a skilled artisan.

In some embodiments, the method described herein comprises administering a fusion protein, composition or vaccine comprising a fusion protein at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99%, or more, sequence identity to a DNAJB1-PRKACA fusion protein. In some embodiments, the fusion protein, composition or vaccine comprises at least 70% sequence identity to SEQ ID NO: 9. In some embodiments, the fusion protein, composition or vaccine comprises at least 90% sequence identity to SEQ ID NO: 9. In some embodiments, the cancer vaccine comprises SEQ ID NO: 9.

In some embodiments, the fusion protein, composition or vaccine induces immune response in a subject expressing a DNAJB1-PRKACA fusion protein.

In some embodiments, the subject has Fibrolamellar hepatocellular carcinoma (FLC), pancreatic cancer, or biliary cancer.

In another aspect, a subject such as a human is identified as suffering from a cancer, such as Fibrolamellar hepatocellular carcinoma (FLC), pancreatic cancer, or biliary cancer, and the identified subject is selected for treatment, and administered a composition or cancer vaccine as disclosed herein.

In some embodiments, the method described herein further comprises administering an adjuvant.

In some embodiments, the method described herein further comprises administering an immune checkpoint inhibitor. Such immune checkpoint inhibitor may include, at least, antibodies or other inhibiting agents to checkpoint inhibitors. In some embodiments, the method described herein comprises administering the vaccine and the immune checkpoint inhibitor simultaneously. In some embodiments, the method described herein comprises administering the vaccine and the immune checkpoint inhibitor sequentially.

In some embodiments, the fusion protein, composition or vaccine induces CD4 and/or CD8 T cell response.

Another aspect of the invention provides for an isolated polynucleotide molecule encoding the fusion protein described herein.

Another aspect of the invention provides for an expression vector comprising the isolated polynucleotide molecule described herein.

Another aspect of the invention provides for a cell comprising the expression vector described herein.

Other aspects of the invention are disclosed infra.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

FIG. 1 is a linear chart comparing CD4⁺ T cell response in Balb-C mice with or without vaccination of the DNAJB1-PRKACA vaccine. The FLC-vaccine was injected on days 0 and 7 into the tail base of 3 Balb-C mice. 14 days after the first injection, mouse T cells were harvested from the spleen and co-cultured with control splenocytes taken from a

non-vaccinated mouse. FLC peptide (SEQ ID NO: 9) or vehicle was added to the co-culture wells and incubated for 24 hours to see if the FLC peptides would be processed and presented by splenocytes and result in activation of T cells taken from vaccinated mice. Activation was measured using intercellular staining of INF γ and flow cytometry.

FIG. 2 is a liner chart comparing CD8⁺ T cell response in Balb-C mice with or without vaccination of the DNAJB1-PRKACA vaccine. The FLC-vaccine was injected on days 0 and 7 into the tail base of 3 Balb-C mice. 14 days after the first injection, mouse T cells were harvested from the spleen and co-cultured with control splenocytes taken from a non-vaccinated mouse. FLC peptide (SEQ ID NO: 9) or vehicle was added to the co-culture wells and incubated for 24 hours to see if the FLC peptides would be processed and presented by splenocytes and result in activation of T cells taken from vaccinated mice. Activation was measured using intercellular staining of INF γ and flow cytometry.

FIGs. 3A-3B are a series of microscopic figures comparing the morphological differences between the FLC-TIBx cell line and its parent TIBx cell line. FIG. 3C is a PCR staining figure showing the presence of the fusion gene within the FLC-TIBx cell line (arrow). The PiggyBac system was used to insert the mouse DNAJB1-PRKACA fusion gene driven by CMV promoter into a TIBx cell line (derived from the cell line BNL 1ME A.7R.1 (ATCC® TIB75™) but passaged in mice to increase aggressiveness). The mouse variant of the DNAJB1-PRKACA fusion gene contains 100% homology to the human DNAJB1-PRKACA fusion gene 12 amino acids upstream and downstream of the fusion event. Cells were single cell sorted and PCRed to identify clones positive for the DNAJB1-PRKACA fusion.

FIG. 4 is a linear chart comparing tumor volume with or without FLC vaccination. Ten mice were injected with 1E6 FLC-TIBx cells. After 3 days, 5 mice were vaccinated with the FLC-peptide AddaVax and Poly(I:C) combination (FLC Vaccine Group) and the other 5 mice were vaccinated with AddaVax and Poly(I:C) alone (Mock Vaccine Group). Ten days after the injection of the FLC-TIBx cells, mice were vaccinated again with either the FLC-peptide AddaVax and Poly(I:C) combination or the AddaVax and Poly(I:C) combination. Tumor volumes and tumor weights were measured comparing the FLC and Mock vaccine groups.

FIG. 5 is a bar chart comparing tumor mass (mg) with or without FLC vaccination, using the same methods as in FIG. 4 described above.

FIGs. 6A and 6B are data showing pre-treatment and on-treatment of a patient receiving a DNAJB1-PRKACA fusion kinase vaccine combined with nivolumab and

ipilimumab. FIG. 6A are images of CT scans for a patient with fibrolamellar hepatocellular carcinoma receiving a DNAJB1-PRKACA fusion kinase peptide vaccine (RKREIFDRYGEEVKEFLAKAKEDF SEQ ID NO: 9) combined with nivolumab and ipilimumab. As compared to the pre-treatment baseline scan, the scans at approximately 16 weeks of therapy demonstrate a marked response to therapy with all visible lesions decreasing in size and enhancement. FIG. 6B is a bar graph showing that the patient's liver enzymes also improved with therapy, consistent with decreasing liver tumor burden. At the time of this on-treatment scan, the patient had a neoantigen-specific response to the DNAJB1-PRKACA fusion by IFN- γ ELISpot Assay, which was not present at study baseline. These findings demonstrate the clinical potential of this therapy in patients with fibrolamellar hepatocellular carcinoma, and the potential of this therapy to induce neoantigen-specific responses against the tumor that may mediate the therapeutic effect.

FIG. 7 are data showing pre-treatment and on-treatment IFN- γ ELISpot Assay for a patient receiving a DNAJB1-PRKACA fusion kinase peptide vaccine combined with nivolumab and ipilimumab. As compared to the pre-treatment ELISpot (Top), the on-treatment ELISpot demonstrates a positive response to both the full 24 amino acid peptide encoding the fusion junction (Pep 9), as well as multiple overlapping 9 amino acid length peptides (RKREIFDRYGEEVKEFLAKAKEDF SEQ ID NO: 9). These findings demonstrate the clinical potential of this therapy to induce neoantigen-specific responses against the tumor that may mediate the therapeutic effect.

DETAILED DESCRIPTION

The invention is based, at least in part, on the surprising discovery that fusion proteins comprising a DNAJB1 portion and a PRKACA portion facilitate effector T cell immune response and can be used as cancer vaccines to treat or prevent cancer. Pediatric liver cancer (e.g., Fibrolamellar Hepatocellular Carcinoma (FLC)) expresses a fusion protein, which joins the J domain of heat shock protein genes (e.g., DNAJB1) to the kinase domain of a cAMP-dependent protein kinase (e.g., PRKACA). A corresponding fusion protein comprising a DNAJB1 portion and a PRKACA portion is capable of inducing T cell immune response for a cancer vaccine therapy to treat or prevent these cancers.

Provided herein are fusion proteins, compositions (including immunogenic compositions) and vaccine compositions for use and methods of treating or preventing cancer in a subject comprising administering a composition or vaccine to the subject comprising a fusion protein comprising a DNAJB1 portion and a PRKACA portion, thereby treating or

preventing the cancer in the subject. Preferably, the methods described herein inhibit the growth or progression of cancer, e.g., a tumor, in a subject. For example, the vaccine compositions and methods described herein inhibit the growth of a tumor by at least 1%, e.g., by at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or 100%.

In other cases, the methods and compositions described herein reduce the size of a tumor by at least 1 mm in diameter, e.g., by at least 2 mm in diameter, by at least 3 mm in diameter, by at least 4 mm in diameter, by at least 5 mm in diameter, by at least 6 mm in diameter, by at least 7 mm in diameter, by at least 8 mm in diameter, by at least 9 mm in diameter, by at least 10 mm in diameter, by at least 11 mm in diameter, by at least 12 mm in diameter, by at least 13 mm in diameter, by at least 14 mm in diameter, by at least 15 mm in diameter, by at least 20 mm in diameter, by at least 25 mm in diameter, by at least 30 mm in diameter, by at least 40 mm in diameter, by at least 50 mm in diameter or more.

The subject is preferably a mammal in need of such treatment, e.g., a subject that has been diagnosed with cancer, e.g., FLC, or a predisposition thereto, i.e., at risk of developing FLC. The mammal is any mammal, e.g., a human, a primate, a mouse, a rat, a dog, a cat, a horse, as well as livestock or animals grown for food consumption, e.g., cattle, sheep, pigs, chickens, and goats. In a preferred embodiment, the mammal is a human.

Modes of administration include intravenous, systemic, oral, rectal, topical, intraocular, buccal, intravaginal, intracisternal, intracerebroventricular, intratracheal, nasal, transdermal, within/on implants, or parenteral routes. The term "parenteral" includes subcutaneous, intrathecal, intravenous, intramuscular, intraperitoneal, or infusion. Intravenous or intramuscular routes are not particularly suitable for long-term therapy and prophylaxis. They could, however, be preferred in emergency situations. Compositions comprising a composition of the invention can be added to a physiological fluid, such as blood. Oral administration can be preferred for prophylactic treatment because of the convenience to the patient as well as the dosing schedule. Parenteral modalities (subcutaneous or intravenous) may be preferable for more acute illness, or for therapy in patients that are unable to tolerate enteral administration due to gastrointestinal intolerance, ileus, or other concomitants of critical illness. Inhaled therapy is also provided.

Any composition or vaccine for the treatment of cancer, e.g., liver cancer, e.g., FLC, is useful in the methods described herein.

For example, the composition or vaccine comprises an isolated fusion protein described herein with or without an adjuvant. The composition or vaccine may also comprise an isolated polynucleotide molecule encoding the fusion protein described herein. The vaccine may also comprise an expression vector comprising the isolated polynucleotide molecule encoding the fusion protein described herein. The vaccine may also comprise a cell comprising the expression vector comprising an isolated polynucleotide encoding the fusion protein described herein. Such vaccine may also be administered prior to, concurrently with, or subsequent to administration of an immune checkpoint inhibitor or another therapy.

In one aspect, the fusion protein, compositions (including immunogenic compositions) or cancer vaccine compositions described herein and/or the immune checkpoint inhibitor is administered at a dosage of 0.01-10 mg/kg (e.g., 0.01, 0.05, 0.1, 0.5, 1, 5, or 10 mg/kg) bodyweight. For example, the fusion protein or cancer vaccine described herein and/or the immune checkpoint inhibitor is administered in an amount of 0.01-30 mg (e.g., 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, or 30 mg) per dose. In another example, the fusion protein or cancer vaccine described herein and/or the immune checkpoint inhibitor is administered in the dose range of 0.1 mg/kg to 10 mg/kg of body weight.

In some cases, the fusion protein or vaccine and/or the immune checkpoint inhibitor is administered twice or more, e.g., 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 15 times, 20 times, 25 times, 30 times, 35 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times or more. For example, the fusion protein or vaccine and/or the immune checkpoint inhibitor is administered at least once per week, e.g., at least twice per week, at least three times per week, at least four times per week, at least five times per week, at least six times per week, at least seven times per week. Alternatively, the fusion protein or vaccine and/or the immune checkpoint inhibitor is administered at least once per day, e.g., at least twice per day, at least every eight hours, at least every four hours, at least every two hours, or at least every hour.

The compositions of the invention (e.g., the cancer vaccine comprising an isolated fusion protein comprising a DNAJB1 portion and a PRKACA portion) are administered for a duration of 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 3 weeks, 4 weeks, five weeks, six weeks, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 2 years, 3 years, 4 years, 5 years or more. For example, the composition of the invention (e.g., the cancer vaccine comprising an isolated fusion protein comprising a DNAJB1 portion and a PRKACA portion) are administered one dose every two weeks for 4 to 6 weeks or until the disease is treated.

Optionally, the fusion protein or vaccine and the immune checkpoint inhibitor are administered simultaneously. Alternatively, the vaccine and the immune checkpoint inhibitor are administered sequentially.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

By “cancer vaccine” is meant any vaccine that either treats existing cancer or prevents development of cancer. Vaccines that treat existing cancer are also known as therapeutic cancer vaccines. Some/many of the vaccines are “autologous”, being prepared from samples taken from the patient, and are specific to that patient. Cancer vaccines also include cancer cells, parts of cells, or pure antigens, which work against viruses. For example, a signature protein specifically expressed by cancer cells, or a fragment thereof, may be used as vaccines to active the host immune system to mount an attack against cancer cells in the body.

Vaccines are often combined with other substances or cells called adjuvants that help boost the immune response even further. Adjuvants are any substance whose admixture into the vaccine composition increases or otherwise modifies the immune response to the mutant peptide. Carriers are scaffold structures, for example a polypeptide or a polysaccharide, to which the neoantigenic peptides, is capable of being associated. Optionally, adjuvants are conjugated covalently or non-covalently to the peptides or polypeptides of the invention.

The ability of an adjuvant to increase the immune response to an antigen is typically manifested by a significant increase in immune-mediated reaction, or reduction in disease symptoms. For example, an increase in humoral immunity is typically manifested by a significant increase in the titer of antibodies raised to the antigen, and an increase in T-cell activity is typically manifested in increased cell proliferation, or cellular cytotoxicity, or cytokine secretion. An adjuvant may also alter an immune response, for example, by changing a primarily humoral or Th response into a primarily cellular, or Th response.

Suitable adjuvants include, but are not limited to aluminium salts, Montanide ISA 206, Montanide ISA 50V, Montanide ISA 50, Montanide ISA-51, Montanide ISA-720, 1018

ISS, Amplivax, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, GM-CSF, IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel® vector system, PLG microparticles, resiquimod, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Aquila's QS21 stimulon (Aquila Biotech, Worcester, Mass., USA) which is derived from saponin, and cytokines may be used.

A vaccine composition according to the present invention may comprise more than one different adjuvants. Furthermore, the invention encompasses a therapeutic composition comprising any adjuvant substance including any of the above or combinations thereof. It is also contemplated that the peptide or polypeptide, and the adjuvant can be administered separately in any appropriate sequence.

A carrier may be present independently of an adjuvant. The function of a carrier can for example be to increase the molecular weight of in particular mutant in order to increase their activity or immunogenicity, to confer stability, to increase the biological activity, or to increase serum half-life. Furthermore, a carrier may aid presenting peptides to T-cells. The carrier may be any suitable carrier known to the person skilled in the art, for example a protein or an antigen presenting cell. A carrier protein could be but is not limited to keyhole limpet hemocyanin, serum proteins such as transferrin, bovine serum albumin, human serum albumin, thyroglobulin or ovalbumin, immunoglobulins, or hormones, such as insulin or palmitic acid. For immunization of humans, the carrier must be a physiologically acceptable carrier acceptable to humans and safe. The carrier may be dextrans for example sepharose.

By “agent” is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

By “control” or “reference” is meant a standard of comparison. As used herein, “changed as compared to a control” sample or subject is understood as having a level that is statistically different than a sample from a normal, untreated, or control sample. Control samples include, for example, cells in culture, one or more laboratory test animals, or one or more human subjects. Methods to select and test control samples are within the ability of those in the art. An analyte can be a naturally occurring substance that is characteristically expressed or produced by the cell or organism (e.g., an antibody, a protein) or a substance produced by a reporter construct (e.g., β -galactosidase or luciferase). Depending on the method used for detection, the amount and measurement of the change can vary.

Determination of statistical significance is within the ability of those skilled in the art, e.g., the number of standard deviations from the mean that constitute a positive result.

As used herein, “detecting” and “detection” are understood that an assay performed for identification of a specific analyte in a sample, e.g., an antigen in a sample or the level of an antigen in a sample. The amount of analyte or activity detected in the sample can be none or below the level of detection of the assay or method.

By “effective amount” is meant the amount of a required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an “effective” amount.

As used herein, the terms “conjugated,” “linked,” “attached,” “fused” and “tethered,” when used with respect to two or more moieties, means that the moieties or domains are physically associated or connected with one another, either directly or via one or more additional moieties that serve as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which the structure is used, e.g., physiological conditions. The linkage can be based on genetic fusion according to the methods known in the art or can be performed by, e.g., chemical cross-linking. The compounds and targeting agents may be linked by a flexible linker, such as a polypeptide linker. The polypeptide linker can comprise plural, hydrophilic or peptide-bonded amino acids of varying lengths. The term “associated” will be used for the sake of brevity and is meant to include all possible methods of physically associating each compound to a targeting ligand.

A “fusion protein” or a “fusion polypeptide” refer to a chimeric protein encoding two or more separate peptide or protein sequences or portions that are recombinantly expressed, linked or chemically synthesized as a single moiety. Each of the portions is a polypeptide having a different property. The property may be a biological property, such as activity in vitro or in vivo. The property may also be simple chemical or physical property, such as binding to a target molecule, catalysis of a reaction, etc. The two portions are in reading frame with each other.

The term “polynucleotide” or “nucleic acid” as used herein designates mRNA, RNA, cRNA, cDNA or DNA. As used herein, a “nucleic acid encoding a polypeptide” is understood as any possible nucleic acid that upon (transcription and) translation would result

in a polypeptide of the desired sequence. The degeneracy of the nucleic acid code is well understood. Further, it is well known that various organisms have preferred codon usage, etc. Determination of a nucleic acid sequence to encode any polypeptide is well within the ability of those of skill in the art.

As used herein, “isolated” or “purified” when used in reference to a polypeptide means that a polypeptide or protein has been removed from its normal physiological environment (e.g., protein isolated from plasma or tissue, optionally bound to another protein) or is synthesized in a non-natural environment (e.g., artificially synthesized in an in vitro translation system or using chemical synthesis). Thus, an “isolated” or “purified” polypeptide can be in a cell-free solution or placed in a different cellular environment (e.g., expressed in a heterologous cell type). The term “purified” does not imply that the polypeptide is the only polypeptide present, but that it is essentially free (about 90-95%, up to 99-100% pure) of cellular or organismal material naturally associated with it, and thus is distinguished from naturally occurring polypeptide. Similarly, an isolated nucleic acid is removed from its normal physiological environment. “Isolated” when used in reference to a cell means the cell is in culture (i.e., not in an animal), either cell culture or organ culture, of a primary cell or cell line. Cells can be isolated from a normal animal, a transgenic animal, an animal having spontaneously occurring genetic changes, and/or an animal having a genetic and/or induced disease or condition. An isolated virus or viral vector is a virus that is removed from the cells, typically in culture, in which the virus was produced

As used herein, “kits” are understood to contain at least one non-standard laboratory reagent for use in the methods of the invention in appropriate packaging, optionally containing instructions for use. The kit can further include any other components required to practice the method of the invention, as dry powders, concentrated solutions, or ready to use solutions. In some embodiments, the kit comprises one or more containers that contain reagents for use in the methods of the invention; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding reagents.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence but will typically exhibit substantial identity. Polynucleotides having “substantial identity” to an endogenous sequence are typically capable of hybridizing with at least one strand of a

double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 .mu.g/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C. in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42 degrees. C. in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred

embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

“Obtaining” is understood herein as manufacturing, purchasing, or otherwise coming into possession of.

As used herein, “operably linked” is understood as joined, preferably by a covalent linkage, e.g., joining an amino-terminus of one peptide, e.g., expressing an enzyme, to a carboxy terminus of another peptide, e.g., expressing a signal sequence to target the protein to a specific cellular compartment; joining a promoter sequence with a protein coding sequence, in a manner that the two or more components that are operably linked either retain their original activity, or gain an activity upon joining such that the activity of the operably linked portions can be assayed and have detectable activity, e.g., enzymatic activity, protein expression activity.

The phrase “pharmaceutically acceptable carrier” is art recognized and includes a pharmaceutically acceptable material, composition or vehicle, suitable for administering compounds of the present invention to mammals. The carriers include liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic

saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, α -tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical, transdermal, buccal, sublingual, intramuscular, intracardiac, intraperitoneal, intrathecal, intracranial, rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound that produces a therapeutic effect.

As used herein, the terms "prevent," "preventing," "prevention," "prophylactic treatment" and the like refer to reducing the probability of developing a disorder or condition in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition.

A "polypeptide" or "peptide" as used herein is understood as two or more independently selected natural or non-natural amino acids joined by a covalent bond (e.g., a peptide bond). A peptide can include 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more natural or non-natural amino acids joined by peptide bonds. Polypeptides as described herein include full length proteins (e.g., fully processed proteins) as well as shorter amino acids sequences (e.g., fragments of naturally occurring proteins or synthetic polypeptide fragments). Optionally the peptide further includes one or more modifications such as modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which 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. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslational natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formulation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, *Proteins, Structure and Molecular Properties*, 2nd ed., T. E. Creighton, W.H. Freeman and Company, New York (1993); *Posttranslational Covalent Modification of Proteins*, B. C. Johnson, ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., *Meth. Enzymol* 182:626-646 (1990); Rattan et al., *Ann. N.Y. Acad. Sci.* 663:48-62 (1992)).

The invention encompasses “fragments” and “peptides” of SEQ ID NOs: 1-9 described herein. Such peptides represent portions of the polypeptide that have, for example, specific immunogenic or binding properties. A fragment can be between 3-10 amino acids, 10-20 amino acids, in length or even longer. Amino acid sequences having at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% identity, and most preferably 95% identity to the fragments described herein are also included within the scope of the present invention.

The term “reduce” or “increase” is meant to alter negatively or positively, respectively, by at least 5%. An alteration may be by 5%, 10%, 25%, 30%, 50%, 75%, or even by 100%.

A “sample” as used herein refers to a biological material that is isolated from its environment (e.g., blood or tissue from an animal, cells, or conditioned media from tissue culture) and is suspected of containing, or known to contain an analyte, such as a protein. A

sample can also be a partially purified fraction of a tissue or bodily fluid. A reference sample can be a “normal” sample, from a donor not having the disease or condition fluid, or from a normal tissue in a subject having the disease or condition. A reference sample can also be from an untreated donor or cell culture not treated with an active agent (e.g., no treatment or administration of vehicle only). A reference sample can also be taken at a “zero time point” prior to contacting the cell or subject with the agent or therapeutic intervention to be tested or at the start of a prospective study.

A “subject” as used herein refers to an organism. In certain embodiments, the organism is an animal. In certain embodiments, the subject is a living organism. In certain embodiments, the subject is a cadaver organism. In certain preferred embodiments, the subject is a mammal, including, but not limited to, a human or non-human mammal. In certain embodiments, the subject is a domesticated mammal or a primate including a non-human primate. Examples of subjects include humans, monkeys, dogs, cats, mice, rats, cows, horses, goats, and sheep. A human subject may also be referred to as a patient.

A “subject sample” can be a sample obtained from any subject, typically a blood or serum sample, however the method contemplates the use of any body fluid or tissue from a subject. The sample may be obtained, for example, for diagnosis of a specific individual for the presence or absence of a particular disease or condition.

A subject “suffering from or suspected of suffering from” a specific disease, condition, or syndrome has a sufficient number of risk factors or presents with a sufficient number or combination of signs or symptoms of the disease, condition, or syndrome such that a competent individual would diagnose or suspect that the subject was suffering from the disease, condition, or syndrome. Methods for identification of subjects suffering from or suspected of suffering from conditions associated with cancer is within the ability of those in the art. Subjects suffering from, and suspected of suffering from, a specific disease, condition, or syndrome are not necessarily two distinct groups.

As used herein, “susceptible to” or “prone to” or “predisposed to” a specific disease or condition and the like refers to an individual who based on genetic, environmental, health, and/or other risk factors is more likely to develop a disease or condition than the general population. An increase in likelihood of developing a disease may be an increase of about 10%, 20%, 50%, 100%, 150%, 200%, or more.

As used herein, the terms “treat,” “treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that,

although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

Ranges provided herein are understood to be shorthand for all of the values within the range.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive.

Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein can be modified by the term about.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

The transitional term "comprising," which is synonymous with "including," "containing," or "characterized by," 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.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from 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 this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the

practice or testing of the present invention, suitable methods and materials are described below. All published foreign patents and patent applications cited herein are incorporated herein by reference. GenBank and NCBI submissions indicated by accession number cited herein are incorporated herein by reference. All other published references, documents, manuscripts and scientific literature cited herein are incorporated herein by reference. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Fibrolamellar Hepatocellular Carcinoma (FLC)

Fibrolamellar Hepatocellular Carcinoma (FLC) is a type of liver cancer. It typically affects young adults and is histologically characterized by laminated fibrous layers interspersed between the tumor cells. This form of cancer is often advanced when diagnosed due to lack of symptoms. FLC does not produce the alpha fetoprotein biomarker, typically observed in hepatocellular carcinoma. However, elevated neurotensin levels have been observed in FLC patients. See reviews of FLC: Mavros et al. (2012) J Am Coll Surg. 215(6):820-30; Chun et al, (2013) Recent Results Cancer Res. 190: 101-10; and Paradis (2013) Recent Results Cancer Res. 190:21-32, each of which are hereby incorporated by reference in their entireties. The exact underlying cause of FLC is poorly understood. Unlike other forms of liver cancer, FLC typically occurs in the absence of underlying liver inflammation or scarring; thus, specific risk factors for this condition remain unidentified. FLC is typically treated with surgical resection. Many people with early FLC have no signs or symptoms of the condition. When present, symptoms are often nonspecific and blamed on other, more common conditions. Signs and symptoms may include: abdominal pain, loss of appetite, weight loss, malaise, jaundice, nausea and/or vomiting, a palpable liver mass found on a physical exam. Other signs and symptoms that have been reported less commonly include migratory thrombophlebitis (Trousseau syndrome) or venous thrombosis, and gynecomastia (excessive breast tissue in males).

Medical imaging techniques, such as computed tomography (CT scan) and endoscopic ultrasound (EUS) are used both to confirm the diagnosis and to help decide whether the tumor can be surgically removed. Magnetic resonance imaging and positron emission tomography may also be used, and magnetic resonance cholangiopancreatography may be useful in some cases. Abdominal ultrasound is less sensitive and will miss small tumors but can identify cancers that have spread to the liver and build-up of fluid in the peritoneal cavity (ascites). A biopsy by fine needle aspiration, often guided by endoscopic ultrasound, may be used where there is uncertainty over the diagnosis. Liver function tests

can show a combination of results indicative of bile duct obstruction (raised conjugated bilirubin, γ -glutamyl transpeptidase and alkaline phosphatase levels).

Chaperone DnaJ, also known as Heat Shock p40 (Hsp40, 40 kD), is a molecular chaperone protein. It protects proteins from aggregation during synthesis and during cellular stress. It consists of three domains: the N-terminal domain comprising the J domain; a central domain comprising a cysteine rich region (zinc-finger domain); and the C-terminal domain which functions in dimerization and chaperoning. Non-limiting examples of proteins containing a J domain include: DNAJA1; DNAJA2; DNAJ A3; DNAJA4; DNAJB1; DNAJB11; DNAJB13; DNAJB4; DNAJB5; MST104. See a review of Chaperone DnaJ proteins: Kakkar et al., (2012) *Curr Top Med Chem*. 12(22):2479-90), which is incorporated by reference in its entirety.

A cAMP-dependent protein kinase comprises a family of protein kinases, and is also known as protein kinase A (PKA). It is an enzyme whose activity is dependent on cellular levels of cyclic AMP (cAMP). cAMP-dependent protein kinase catalytic subunit alpha (PRKACA), a member of the family, is an enzyme that in humans is encoded by the PRKACA gene. See for example, Taylor et al, (2013) *Biochim Biophys Acta*. 1834(7): 1271-8), which is incorporated by reference in its entirety.

Recently, a DNAJB1-PRKACA fusion protein was discovered in FLC patients. See Honeyman et al., *Science*, 343(6174), pp 1010-1014 (February 2014).

Pharmaceutical Compositions and Administration

The present invention comprises pharmaceutical preparations comprising a vaccine (e.g., a fusion protein comprising a DNAJB1 portion and a PRKACA portion) together with a pharmaceutically acceptable carrier. Such compositions are useful for the treatment or prevention of cancer, e.g., FLC. Fusion proteins of the invention may be administered as part of a pharmaceutical composition. The compositions should be sterile and contain a therapeutically effective amount of the polypeptides in a unit of weight or volume suitable for administration to a subject.

As used herein, the terms “pharmaceutically acceptable”, “physiologically tolerable” and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a mammal.

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline,

dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

The therapeutic composition of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethyl amine, 2-ethylamino ethanol, histidine, procaine and the like. Particularly preferred are the salts of TFA and HCl.

Physiologically tolerable carriers are well known in the art. Exemplary of liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes.

Liquid compositions also can contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions.

These compositions can be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10 mL vials are filled with 5 mL of sterile-filtered 1% (w/v) aqueous polypeptide solution, and the resulting mixture can then be lyophilized. The infusion solution can be prepared by reconstituting the lyophilized material using sterile Water-for-Injection (WFI).

The compositions can be administered in effective amounts. The effective amount will depend upon the mode of administration, the particular condition being treated, and the desired outcome. It may also depend upon the stage of the condition, the age and physical condition of the subject, the nature of concurrent therapy, if any, and like factors well known to the medical practitioner. For therapeutic applications, it is that amount sufficient to achieve a medically desirable result.

The dosage ranges for the administration of the polypeptide vary. In general, amounts are large enough to produce the desired effect in which disease symptoms of a cancer, e.g., FLC, are ameliorated. The dosage should not be so large as to cause adverse side effects. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage also can be adjusted by the individual physician in the event of any complication.

A therapeutically effective amount is an amount sufficient to produce a measurable inhibition of symptoms of a condition (e.g., a reduction in tumor size or increase in subject survival time). Such symptoms are measured in conjunction with assessment of related clinical parameters.

A therapeutically effective amount of a fusion protein or cancer vaccine of this invention in the form of a fusion protein or polypeptide, or fragment thereof, is typically an amount of protein or polypeptide such that when administered in a physiologically tolerable composition is sufficient to achieve a plasma concentration of from about 0.1 microgram (ug) per milliliter (mL) to about 200 ug/mL, or from about 1 ug/mL to about 150 ug/mL. In one embodiment, the plasma concentration in molarity is from about 2 micromolar (uM) to about 5 millimolar (mM) or from 100 uM to 1 mM Cthrc1 polypeptide. In other embodiments, the doses of polypeptide ranges from about 500mg/Kg to about 1.0g/kg (e.g., 500, 600, 700, 750, 800, 900, 1000 mg/kg).

The agents of the invention can be administered parenterally by injection or by gradual infusion over time. In other embodiments, agents are administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, transdermally, topically, intraocularly, orally, intranasally, and can be delivered by peristaltic means. In one embodiment, a therapeutic composition containing an agent of this invention are administered in a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered and timing depends on the patient to be treated, capacity of the patient's system to utilize the active ingredient, and degree of therapeutic effect desired. Precise amounts of active ingredient required to be administered depend on the judgement of the practitioner and are

peculiar to each individual. However, suitable dosage ranges for systemic application are disclosed herein and depend on the route of administration. Suitable regimes for administration also are variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations in the blood in the ranges specified for *in vivo* therapies are contemplated.

Therapy

As demonstrated herein, a single therapy comprising a fusion protein or vaccine (e.g., a fusion protein comprising a DNAJB1 portion and a PRKACA portion) or a combinational therapy comprising a fusion protein or vaccine (e.g., a fusion protein comprising a DNAJB1 portion and a PRKACA portion) and an immune checkpoint inhibitor is useful for the treatment or prevention of cancer (e.g., FLC).

Therapy may be provided wherever therapy for these conditions is performed: at home, the doctor's office, a clinic, a hospital's outpatient department, or a hospital. Treatment generally begins at a hospital so that the doctor can observe the therapy's effects closely and make any adjustments that are needed. The duration of the therapy depends on the kind of disease being treated, the age and condition of the patient, the stage and type of the patient's disease, and how the patient's body responds to the treatment. Drug administration may be performed at different intervals (e.g., daily, weekly, or monthly). Therapy may be given in on-and-off cycles that include rest periods so that the patient's body has a chance to build healthy new cells and regain its strength.

A combination comprising a fusion protein or vaccine (e.g., a fusion protein comprising a DNAJB1 portion and a PRKACA portion) and an immune checkpoint inhibitor may be administered within a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the compounds to patients suffering from a disease that is associated with a metabolic syndrome. Administration may begin before the patient is symptomatic. Any appropriate route of administration may be employed, for example, administration may be topical, parenteral, intravenous, intraarterial, subcutaneous, intratumoral, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intrahepatic, intracapsular, intrathecal, intracisternal, intraperitoneal, intranasal, aerosol, suppository, or oral administration. For example, therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the

form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found, for example, in "Remington: The Science and Practice of Pharmacy" Ed. A. R. Gennaro, Lippincourt Williams & Wilkins, Philadelphia, Pa., 2000. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

The formulations can be administered to human patients in therapeutically effective amounts (e.g., amounts which prevent, eliminate, or reduce a pathological condition) to provide therapy for a disease or condition. "Therapeutically effective amount" is intended to include an amount of a compound useful in the present invention or an amount of the combination of compounds claimed, e.g., to treat or prevent the disease or disorder, or to treat the symptoms of the disease or disorder, in a host. The combination of compounds is preferably a synergistic combination. Synergy, as described for example by Chou and Talalay, *Adv. Enzyme Regul.* 22:27-55 (1984), occurs when the effect of the compounds when administered in combination is greater than the additive effect of the compounds when administered alone as a single agent. In general, a synergistic effect is advantageously demonstrated at suboptimal concentrations of the compounds. Synergy can be in terms of lower cytotoxicity, increased activity, or some other beneficial effect of the combination compared with the individual components. If desired, treatment with an agent of the invention may be combined with therapies for the treatment of PDA.

Immune checkpoint inhibitors described herein

Suitable immune checkpoint inhibitors comprise an inhibitor of CTLA-4, PD-1, PDL-1, Lag3, LAIR1, or LAIR. For example, the immune checkpoint inhibitor comprises a CTLA-4 antibody, a PD-1 antibody, a PDL-1 antibody, a Lag3 antibody, a LAIR1 antibody, or a LAIR 2 antibody. Additional immune checkpoint inhibitors include Interferon

(Interferon Alfa-2B), Pembrolizumab, atezolizumab, durvalumab, LAG3 (Lymphocyte-activation gene 3), TIGIT (T cell immunoreceptor with Ig and ITIM domains), 41BB (CD137 or tumor necrosis factor receptor superfamily member 9 (TNFRSF9), ICOSL, or CD40 (cluster of differentiation 40). Furthermore, TIM3 (T-cell immunoglobulin and mucin-domain containing-3) and OX40 are also contemplated. In other examples, recombinant cytokines such as IL-2 (interleukin 2), IL-12 (interleukin 12) and IL-18 (interleukin 18) are used. Exemplary immune checkpoint inhibitors are commercially available and have been developed by Abcam, Cell Signaling Technology, R&D Systems and BioXCell.

The table below shows FDA approved checkpoint inhibitors (taken from *Ther Adv Respir Dis* 2018, Vol. 12: 1–13).

Table 1. Timeline for FDA approval of checkpoint inhibitors.

Drug	Manufacturer	FDA approval	Indication	Companion diagnostic
Nivolumab	Bristol-Myers Squibb (Princeton, New Jersey)	March 2015	Second-line advanced stage NSCLC (squamous cell carcinoma)	None required
Nivolumab	Bristol-Myers Squibb	October 2015	Second-line advanced stage NSCLC (nonsquamous cell carcinoma)	None required
Pembrolizumab	Merck (Kenilworth, New Jersey)	October 2015	Second-line advanced stage NSCLC	PD-L1 IHC >1% TPS*
Atezolizumab	Genentech/Roche (San Francisco, California)	April 2016	Second-line advanced stage NSCLC	None required
Pembrolizumab	Merck	October 2016	First-line advanced stage NSCLC	PD-L1 IHC >50% TPS
Pembrolizumab with carboplatin/ pemetrexed	Merck	May 2017	First-line advanced stage NSCLC (nonsquamous cell carcinoma)	None required

FDA, US Food and Drug Administration; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; PD-1, programmed cell death 1; PD-L1 programmed cell death ligand 1; TPS, tumor proportion score.

Kits

The invention provides kits for the treatment or prevention of a cancer, e.g., a FLC. In some embodiments, the kit includes a therapeutic or prophylactic composition containing an effective amount of an agent described herein. In some embodiments, the kit comprises a sterile container that contains a therapeutic or prophylactic composition; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

If desired an agent of the invention is provided together with instructions for administering the agent to a subject having or at risk of developing a cancer. The instructions will generally include information about the use of the composition for the treatment or prevention of a cancer. In other embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of a cancer or symptoms thereof; precautions; warnings; indications; counter-indications; overdose information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

This invention is further illustrated by the following examples, which should not be construed as limiting. All documents mentioned herein are incorporated herein by reference.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

Example 1:

Fibrolamellar hepatocellular carcinoma (FLC) is a rare and often lethal form of liver cancer that typically affects adolescents and young adults without underlying cirrhosis. There is no standardized systemic therapy for this cancer, and patients with unresectable disease have a median survival of only 12 months. A chimeric transcript between DNAJB1, a homolog of the molecular chaperone DNAJ, and PRKACA, the catalytic domain of protein kinase A, was recently identified as the signature genetic event initiating FLC. In the vast majority of cases, the fusion occurs within the intron such that DNAJB1 exon 1 is fused in frame with the beginning of PRKACA exon 2. This fusion has also been identified in other cancers, including pancreatic and biliary cancers. This fusion kinase may be a therapeutic opportunity for neoantigen-specific immunotherapy. Here, it was tested for treating FLC and other cancers with an exemplary cancer vaccine against the DNAJB1-PRKACA fusion protein.

Human DNAJB1 protein isoform 1 (SEQ ID NO: 1):

MGKDYYQTLGLARGASDEEIKRAYRRQALRYHPDKNKEPGAEEKFKEIAE
AYDVLS DPRKREIFDRYGEEGLKGSGPSGGSGGGANGTSFSYTFHGDPHA
MFAEFFGGRNPFDTFFGQRNGEEGMDIDDPFSGFPMGMGGFTNVNFGSR
SAQEPARKKQDPPVTHDLRVSLEEIYSGCTKKMKISHKRLNPDGKSIRNE

DKILTIEVKKGWKEGTKITFPKEGDQTSNNIPADIVFVLKDKPHNIFKRD
 GSDVIYPARISLREALCGCTVNVPTLDGRTIPVVFKDVIRPGMRRKVPGE
 GLPLPKTPEKRGDLIIEFEVIFPERIPQTSRTVLEQVLPI

Human DNAJB1 protein isoform 2 (SEQ ID NO: 2):

MFAEFFGGRNPFDTFFGQRNGEEGMDIDDPFSGFPMGMGGFTNVNFGSR
 SAQEPARKKQDPPVTHDLRVSLLEIYSGCTKKMKISHKRLNPDGKSIRNE
 DKILTIEVKKGWKEGTKITFPKEGDQTSNNIPADIVFVLKDKPHNIFKRD
 GSDVIYPARISLREALCGCTVNVPTLDGRTIPVVFKDVIRPGMRRKVPGE
 GLPLPKTPEKRGDLIIEFEVIFPERIPQTSRTVLEQVLPI

Human PRKACA protein isoform 1 (SEQ ID NO: 3):

MGNAAAAKKG SEQESVKEFL AKAKEDFLKK WESPAQNTAH LDQFERIKTL
 GTGSFGRVML VKHKETGNHY AMKILDKQKV VKLKQIEHTL NEKRILQAVN
 FPFLVKLEFS FKDNSNLYMV MEYVPGGEMF SHLRRIGRFS EPHARFYAAQ
 IVLTFEYLHS LDLIYRDLKP ENLLIDQQGY IQVTDFGFAK RVKGRTWTLC
 GTPEYLAPEI ILSKGYNKAV DWWALGVLIY EMAAGYPPFF ADQPIQIYEK
 IVSGKVRFPS HFSSDLKDLL RNLLQVDLTK RFGNLKNGVN DIKNHKWFAT
 TDWIAIYQRK VEAPFIPKFK GPGDTSNFDD YEEEEIRVSI NEKCGKEFSE F

Human PRKACA protein isoform 2 (SEQ ID NO: 4):

MASNSSDVKE FLAKAKEDFL KKWESPAQNT AHLQFERIK TLGTGSFGRV
 MLVKHKETGN HYAMKILDKQ KVVKLKQIEH TLNEKRILQA VNFPLVKLE
 FSFKDNSNLY MVMEYVPGGE MFSHLRRIGR FSEPHARFYA AQIVLTFEYL
 HSLDLIYRDL KPENLLIDQQ GYIQVTDFGF AKRVKGRTWT LCGTPEYLAP
 EIILSKGYNK AVDWWALGVL IYEMAAGYPP FFADQPIQIY EKIVSGKVRF
 PSHFSSDLKD LLRNLLQVDL TKRFGNLKNG VNDIKNHKWF ATTDWIAIYQ
 RKVEAPFIPK FKGPGDTSNF DDYEEEEIRV SINEKCGKEF SEF

This exemplary vaccine approach exploits the unique biology of FLC and other cancers with this same fusion gene.

The splice site of the DNAJB1-PRKACA fusion almost always occurs within the intron, and therefore the sequence of the fusion is shared by nearly all patients with FLC. This allows a single “off the shelf” neoantigen-specific vaccine to be utilized in patients with this cancer as well as other cancers harboring this same fusion gene. In the sequencing data provided in Catalogue of Somatic Mutations in Cancer (COSMIC), approximately 93% of patients with FLC have an inferred breakpoint within the intron resulting in a conserved splicing of the coding regions of DNAJB1 exon 1 and PRKACA exon 2.

Human DNAJB1 protein isoform 1 exon 1

(SEQ ID NO: 5):

MGKDYYQTLGLARGASDEEIKRAYRRQALRYHPDKNKEPGAEKFKIEIAEAYDVLS
DPRKREIFDRYGEE

Human PRKACA protein isoform 1 exon 2

(SEQ ID NO: 6):

VKEFLAKAKEDFLKKWESPAQ

Although alternative splicing junctions have been described in rare cases, low levels of 3' to exon 1 of DNAJB1 and 5' to exon 2 of PRKACA splicing may also occur in such cases due to alternative tumor splicing events.

The DNAJB1-PRKACA fusion is a target for the immune system

To determine if a vaccine against the DNAJB1-PRKACA fusion is feasible, an exemplary peptide vaccine was created to contain 12 amino acids from the DNAJB1 corresponding to the amino acids found directly upstream of the DNAJB1-PRKACA fusion (RKREIFDRYGEE, SEQ ID NO: 7) and 12 amino acids from PRKACA corresponding to the amino acids found directly downstream of the DNAJB1-PRKACA fusion (VKEFLAKAKEDF, SEQ ID NO: 8). This fusion peptide (RKREIFDRYGEEVKEFLAKAKEDF, SEQ ID NO: 9) was combined with AddaVax (Invivogen) and Poly(I:C) (Invivogen). In this example, the combination of a peptide containing the DNAJB1-PRKACA junction plus an adjuvant is referred as the FLC-Vaccine. In other examples, the peptide containing the DNAJB1-PRKACA junction itself is referred to the FLC-Vaccine.

In an exemplary experiment, Balb-C mice were vaccinated with the DNAJB1-PRKACA vaccine. Specifically, the FLC-vaccine was injected on days 0 and 7 into the tail base of 3 Balb-C mice. Fourteen days after the first injection, mouse T cells were harvested from the spleen and co-cultured with control splenocytes taken from a non-vaccinated mouse. FLC peptide or vehicle was added to the co-culture wells and incubated for 24 hours to see if the FLC peptides would be processed and presented by splenocytes and result in activation of T cells taken from vaccinated mice. Activation was measured using intercellular staining of INF γ and flow cytometry. As shown in FIGs. 1 and 2, vaccination of the Balb-C mice with the DNAJB1-PRKACA vaccine generated CD4 and CD8 T cells response against the DNAJB1-PRKACA fusion construct.

The FLC-vaccine has anti-tumor activity against a DNAJB1-PRKACA driven cancer *in vivo*

To test the effectiveness of the FLC-vaccine *in vivo*, an exemplary mouse model of FLC was created and the PiggyBac system was used to insert the mouse DNAJB1-PRKACA fusion gene driven by CMV promoter into a TIBx cell line (derived from the cell line BNL 1ME A.7R.1 (ATCC® TIB75™), but passaged in mice to increase aggressiveness). The mouse variant of the DNAJB1-PRKACA fusion gene contains 100% homology to the human DNAJB1-PRKACA fusion gene 12 amino acids upstream and downstream of the fusion event. Cells were single cell sorted and PCRed for identify clones positive for the DNAJB1-PRKACA fusion. This process was used to generate the FLC-TIBx cell line.

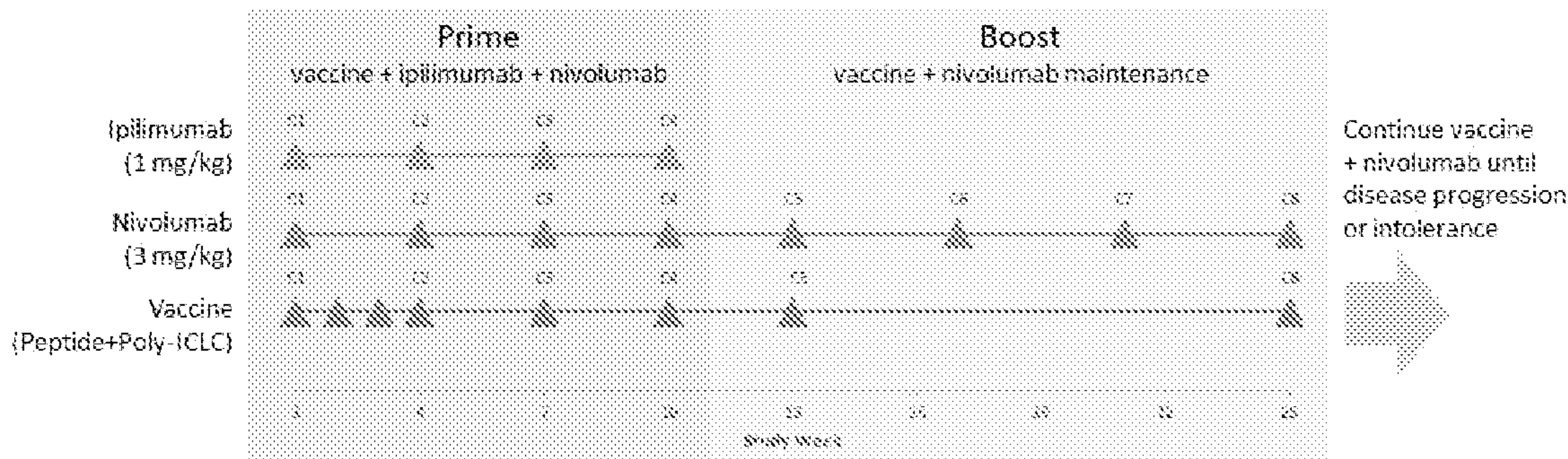
Ten mice were injected with 1E6 FLC-TIBx cells. After 3 days, 5 mice were vaccinated with the FLC-peptide AddaVax and Poly(I:C) combination (FLC Vaccine Group) and the other 5 mice were vaccinated with AddaVax and Poly(I:C) alone (Mock Vaccine Group). 10 days after the injection of the FLC-TIBx cells, mice were vaccinated again with either the FLC-peptide AddaVax and Poly(I:C) combination or the AddaVax and Poly(I:C) combination. Tumor volumes and tumor weights were measured comparing the FLC and Mock vaccine groups.

FIGs. 3A and 3B show the morphological differences between the FLC-TIBx cell line and its parent TIBx cell line. FIG. 3C confirms the presence of the fusion gene within the FLC-TIBx cell line.

FIGs. 4 and 5 show that the FLC peptide significantly delayed the growth of the FLC-TIBx cell line both in terms of tumor volume and tumor weight when tumors were resected at week 24 after implantation.

Clinical effectiveness of a vaccine against the DNAJB1-PRKACA fusion in combination with immune checkpoint inhibitors

In an exemplary human clinical trial, vaccine and various immune checkpoint inhibitors are used with the below exemplary dosing schedule.



The human FLC vaccine consists of 0.3 mg of FLC peptide (RKREIFDRYGEEVKEFLAKAKEDF SEQ ID NO: 9) admixed with 0.5 mg of an adjuvant (e.g., polyinosinic-polycytidylic acid (Poly-ICLC)). An exemplary dosage chart is given below.

<i>Agent</i>	<i>Dose & Schedule</i>	<i>Route</i>
DNAJB1-PRKACA Peptide Vaccine with poly-ICLC adjuvant	PRIME: 0.3 mg peptide + 0.5 mg Poly-ICLC, on Day 1, 8 and 15 of cycle 1, and on day 1 of cycle 2, 3 and 4 (priming phase). BOOST: 0.3 mg peptide + 0.5 mg Poly-ICLC, every 3 cycles (Q12W) beginning from C5D1	3 SC injections (approximately 1 ml each)
Nivolumab	PRIME: 3 mg/kg Q3W* BOOST: 480mg (flat dose) Q4W*	IV over 30 minutes
Ipilimumab	PRIME: 1 mg/kg Q3W for 4 doses*	IV over 30 minutes

Example 2: Combination therapy

FIGs. 6A and 6B are data showing pre-treatment and on-treatment of a patient receiving a DNAJB1-PRKACA fusion kinase vaccine combined with nivolumab and ipilimumab. FIG. 6A are images of CT scans for a patient with fibrolamellar hepatocellular carcinoma receiving a DNAJB1-PRKACA fusion kinase peptide vaccine combined with nivolumab and ipilimumab. As compared to the pre-treatment baseline scan, the scans at approximately 16 weeks of therapy demonstrate a marked response to therapy with all visible lesions decreasing in size and enhancement. FIG. 6B is a bar graph showing that the patient's liver enzymes also improved with therapy, consistent with decreasing liver tumor burden. At the time of this on-treatment scan, the patient had a neoantigen-specific response to the DNAJB1-PRKACA fusion by IFN- γ ELISpot Assay, which was not present at study baseline. These findings demonstrate the clinical potential of this therapy in patients with fibrolamellar hepatocellular carcinoma, and the potential of this therapy to induce neoantigen-specific responses against the tumor that may mediate the therapeutic effect.

FIG. 7 are data showing pre-treatment and on-treatment IFN- γ ELISpot Assay for a patient receiving a DNAJB1-PRKACA fusion kinase peptide vaccine combined with nivolumab and ipilimumab. As compared to the pre-treatment ELISpot (Top), the on-

treatment ELISpot demonstrates a positive response to both the full 24 amino acid peptide encoding the fusion junction (SEQ ID NO: 9), as well as multiple overlapping 9 amino acid length peptides. These findings demonstrate the clinical potential of this therapy to induce neoantigen-specific responses against the tumor that may mediate the therapeutic effect.

Any suitable concentration range for the immune checkpoint inhibitors (e.g., nivolumab and ipilimumab) may be used. Exemplary dosages include about 1-4 mg/kg, about 1-3 mg/kg, about 2-3 mg/kg or about 3 mg/kg. These dosages may be administered during the priming phase or maintenance phase, and may be administered every 3 weeks (Q3W), for four doses. Moreover, during the maintenance phase the immune checkpoint inhibitor may be administered at a flat dose, e.g., from about 100-400 mg/kg, about 100 mg/kg, about 200 mg/kg, about 300 mg/kg, about 400 mg/kg, about 500 mg/kg, or about 480 mg/kg. This dosage may be administered during the maintenance phase every four weeks. A patient may be on therapy at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years or more.

Exemplary immune checkpoint inhibitors include programmed death 1 (PD-1), programmed death ligand 1 (PDL-1), Interferon (Interferon Alfa-2B), Pembrolizumab, atezolizumab, durvalumab, LAG3 (Lymphocyte-activation gene 3), TIGIT (T cell immunoreceptor with Ig and ITIM domains), 41BB (CD137 or tumor necrosis factor receptor superfamily member 9 (TNFRSF9), ICOSL, or CD40 (cluster of differentiation 40). Furthermore, TIM3 (T-cell immunoglobulin and mucin-domain containing-3) and OX40 are also contemplated. In other examples, recombinant cytokines such as IL-2 (interleukin 2), IL-12 (interleukin 12) and IL-18 (interleukin 18) are used.

OTHER EMBODIMENTS

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. Genbank and NCBI submissions indicated by accession number cited herein are hereby

incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

What is claimed is:

1. An isolated fusion protein comprising a DNAJB1 portion and a PRKACA portion.
2. The fusion protein of claim 1, wherein the DNAJB1 portion comprises at least 70% sequence identity to SEQ ID NO: 1, 2, 5, or 7.
3. The fusion protein of claim 1 or 2, wherein the DNAJB1 portion comprises at least 90% sequence identity to SEQ ID NO: 1, 2, 5, or 7.
4. The fusion protein of any preceding claim, wherein the DNAJB1 portion comprises SEQ ID NO: 1, 2, 5, or 7.
5. The fusion protein of any preceding claim, wherein the PRKACA portion comprises at least 70% sequence identity to SEQ ID NO: 3, 4, 6, or 8.
6. The fusion protein of any preceding claim, wherein the PRKACA portion comprises at least 90% sequence identity to SEQ ID NO: 3, 4, 6, or 8.
7. The fusion protein of any preceding claim, wherein the PRKACA portion comprises SEQ ID NO: 3, 4, 6, or 8.
8. The fusion protein of any preceding claim, comprising SEQ ID NO: 9.
9. The fusion protein of any preceding claim, wherein the DNAJB1 portion comprises SEQ ID NO: 1 fused to a PRKACA portion comprising SEQ ID NOS: 3, 4, 6, or 8.
10. The fusion protein of any preceding claim, wherein the DNAJB1 portion comprises SEQ ID NO: 2 fused to a PRKACA portion comprising SEQ ID NOS: 3, 4, 6, or 8.
11. The fusion protein of any preceding claim, wherein the DNAJB1 portion comprises SEQ ID NO: 5 fused to a PRKACA portion comprising SEQ ID NOS: 3, 4, 6, or 8.

12. The fusion protein of any preceding claim, wherein the DNAJB1 portion comprises SEQ ID NO: 7 fused to a PRKACA portion comprising SEQ ID NOS: 3, 4, 6, or 8.
13. The fusion protein of any preceding claim, wherein one or more DNAJB1 sequences are fused to two or more PRKACA sequences.
14. The fusion protein of any preceding claim, wherein two or more DNAJB1 sequences are fused to two or more PRKACA sequences.
15. The fusion protein of any preceding claim, wherein two or more DNAJB1 sequences are fused to two or more PRKACA sequences.
16. The fusion protein of any preceding claim, comprising tandem repeats of a DNAJB1 portion fused to a PRKACA portion.
17. The fusion protein of any preceding claim, capable of inducing immune response in a subject expressing a DNAJB1-PRKACA fusion protein.
18. An immunogenic composition comprising a fusion protein or any one of claims 1 through 17.
19. A cancer vaccine comprising a fusion protein or immunogenic composition of any one of claims 1 through 18.
20. A cancer vaccine comprising an isolated fusion protein comprising a DNAJB1 portion and a PRKACA portion.
21. The cancer vaccine of claim 20, wherein the DNAJB1 portion comprises at least 70% sequence identity to SEQ ID NO: 1, 2, 5, or 7.
22. The cancer vaccine of claim 20 or 21, wherein the DNAJB1 portion comprises at least 90% sequence identity to SEQ ID NO: 1, 2, 5, or 7.

23. The cancer vaccine of any one of claims 20 through 22, wherein the DNAJB1 portion comprises SEQ ID NO: 1, 2, 5, or 7.
24. The cancer vaccine of any one of claims 20 through 23, wherein the PRKACA portion comprises at least 70% sequence identity to SEQ ID NO: 3, 4, 6, or 8.
25. The cancer vaccine of any one of claims 20 through 24, wherein the PRKACA portion comprises at least 90% sequence identity to SEQ ID NO: 3, 4, 6, or 8.
26. The cancer vaccine of any one of claims 20 through 25, wherein the PRKACA portion comprises SEQ ID NO: 3, 4, 6, or 8.
27. The cancer vaccine of any one of claims 20 through 26, comprising SEQ ID NO: 9.
28. The cancer vaccine of any one of claims 20 through 27, capable of inducing immune response in a subject expressing a DNAJB1-PRKACA fusion protein.
29. The cancer vaccine of any one of claims 20 through 28, further comprising an adjuvant.
30. The cancer vaccine of any one of claims 20 through 29 preceding claim, further comprising an immune checkpoint inhibitor.
31. The cancer vaccine of any one of claims 20 through 30, capable of inducing CD4 and/or CD8 T cell response.
32. A method of treating or preventing cancer in a subject comprising:
administering to the subject an effective amount of a fusion protein, immunogenic composition or vaccine of any one of claims 1 through 31;
thereby treating or preventing said cancer in said subject.
33. The method of claim 32, wherein the subject has Fibrolamellar hepatocellular carcinoma (FLC), pancreatic cancer, or biliary cancer.

34. The method of claim 32 or 33, wherein the subject is identified as suffering from Fibrolamellar hepatocellular carcinoma (FLC), pancreatic cancer, or biliary cancer, and the fusion protein, composition or vaccine is administered to the identified subject.
35. The method of any one of claims 32-345 further comprising administering an immune checkpoint inhibitor to the subject.
36. The method of claim 35, wherein the 1) fusion protein, composition or vaccine said the 2) immune checkpoint inhibitor are administered simultaneously.
37. The method of claim 35, wherein the 1) fusion protein, composition or vaccine said the 2) immune checkpoint inhibitor are administered sequentially.
38. A method of treating or preventing cancer in a subject comprising:
administering a fusion protein vaccine to said subject;
thereby treating or preventing said cancer in said subject,
wherein the fusion protein or vaccine comprises an isolated fusion protein comprising a DNAJB1 portion and a PRKACA portion.
39. The method of claim 38, wherein the DNAJB1 portion comprises at least 70% sequence identity to SEQ ID NO: 1, 2, 5, or 7.
40. The method of claim 38 or 39, wherein the DNAJB1 portion comprises at least 90% sequence identity to SEQ ID NO: 1, 2, 5, or 7.
41. The method of any one of claims 38-40, wherein the DNAJB1 portion comprises SEQ ID NO: 1, 2, 5, or 7.
42. The method of any one of claims 38-41, wherein the PRKACA portion comprises at least 70% sequence identity to SEQ ID NO: 3, 4, 6, or 8.
43. The method of any one of claims 38-42, wherein the PRKACA portion comprises at least 90% sequence identity to SEQ ID NO: 3, 4, 6, or 8.

44. The method of any one of claims 38-43, wherein the PRKACA portion comprises SEQ ID NO: 3, 4, 6, or 8.
45. The method of any one of claims 38-44, wherein the isolated fusion protein comprises SEQ ID NO: 9.
46. The method of any one of claims 38-45, wherein the fusion protein or vaccine induces immune response in the subject.
47. The method of any one of claims 38-46, wherein the subject expresses a DNAJB1-PRKACA fusion protein.
48. The method of any one of claims 38 through 47, wherein the subject has fibrolamellar hepatocellular carcinoma (FLC), pancreatic cancer, or biliary cancer.
49. The method of any one of claims 38-48, wherein the fusion protein or vaccine further comprises an adjuvant.
50. The method of any one of claims 38-49, further comprising administering an immune checkpoint inhibitor to the subject.
51. The method of claim 50, wherein the 1) vaccine or fusion protein and 2) immune checkpoint inhibitor are administered simultaneously.
52. The method of claim 50, wherein the 1) vaccine or fusion protein and 2) immune checkpoint inhibitor are administered sequentially.
53. The method of any one of claims 38-52, wherein the vaccine or fusion protein induces CD4 and/or CD8 T cell response.
54. An isolated polynucleotide molecule encoding the fusion protein of any one of claims 1-17.
55. An expression vector comprising the isolated polynucleotide molecule of claim 54.

56. A cell comprising the expression vector of claim 55.

FIG. 1

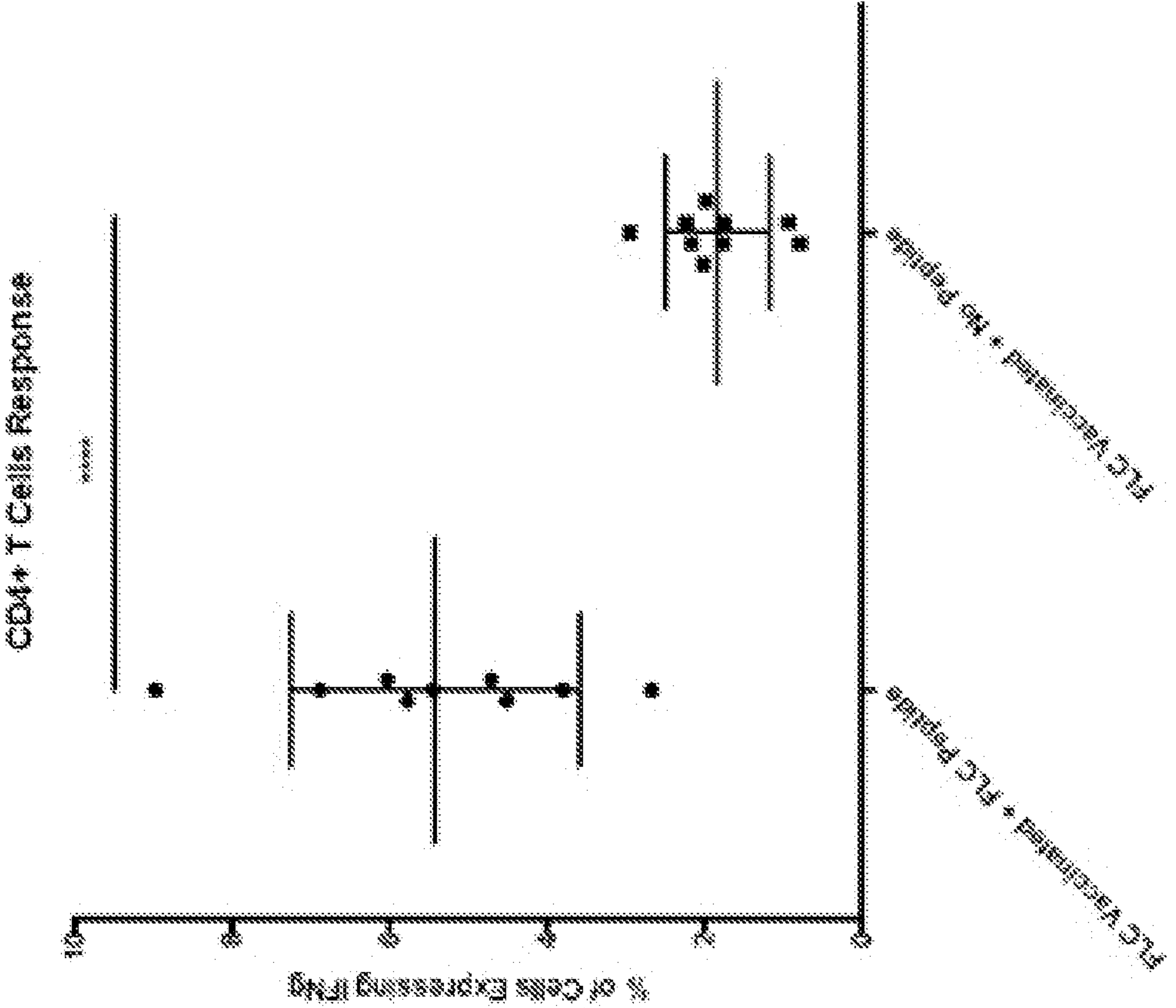


FIG. 2

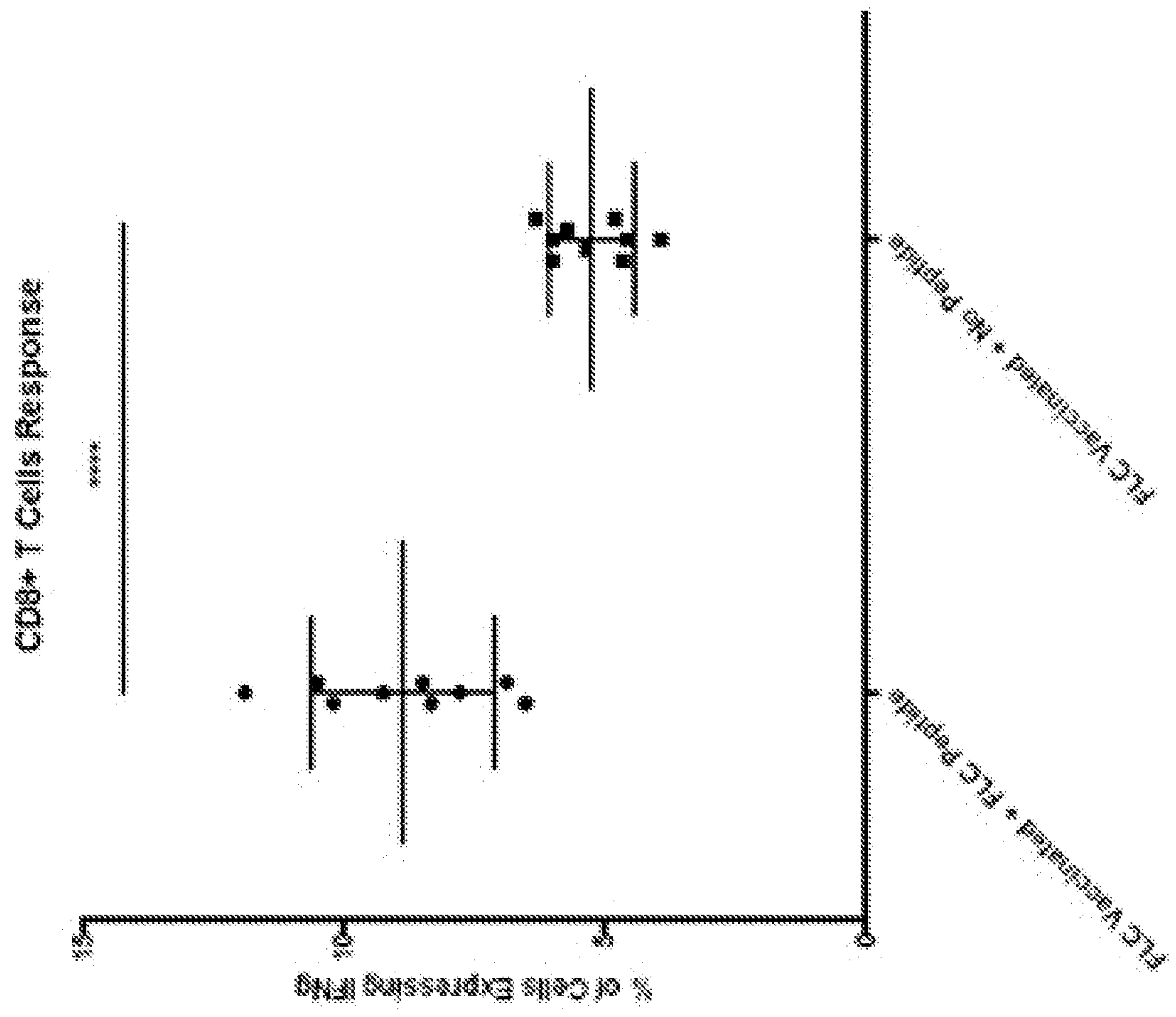


FIG. 3C

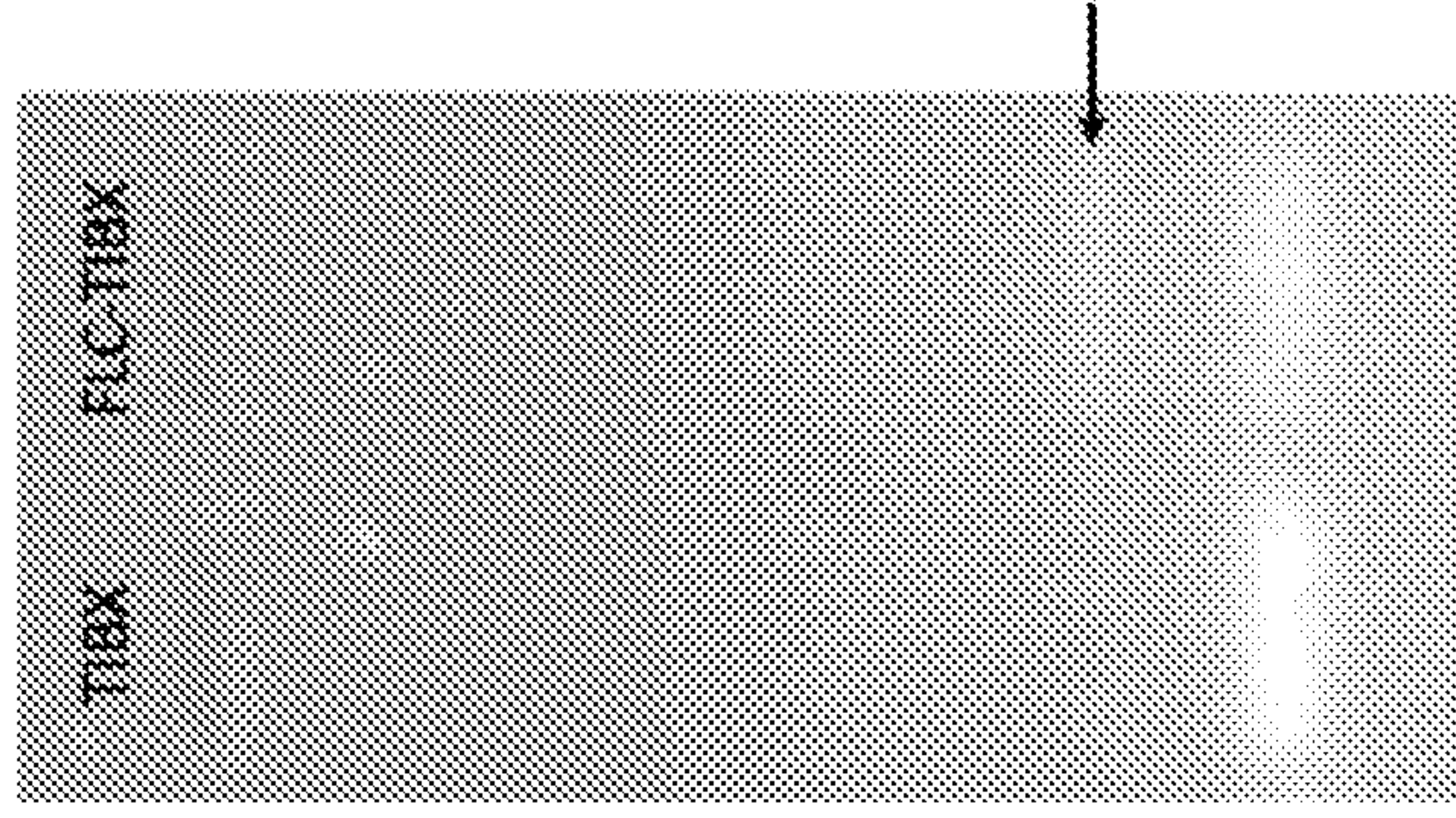


FIG. 3B

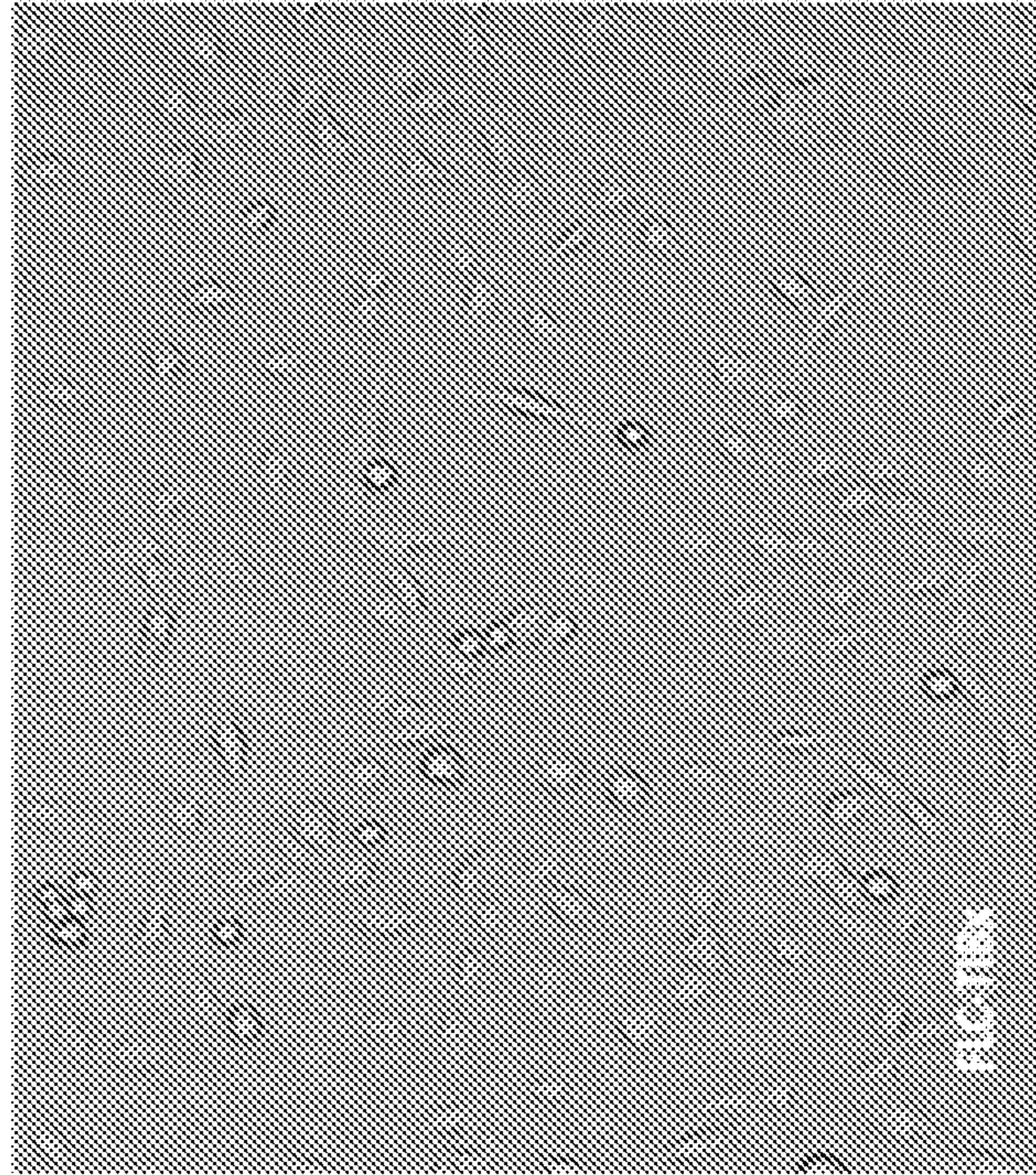


FIG. 3A

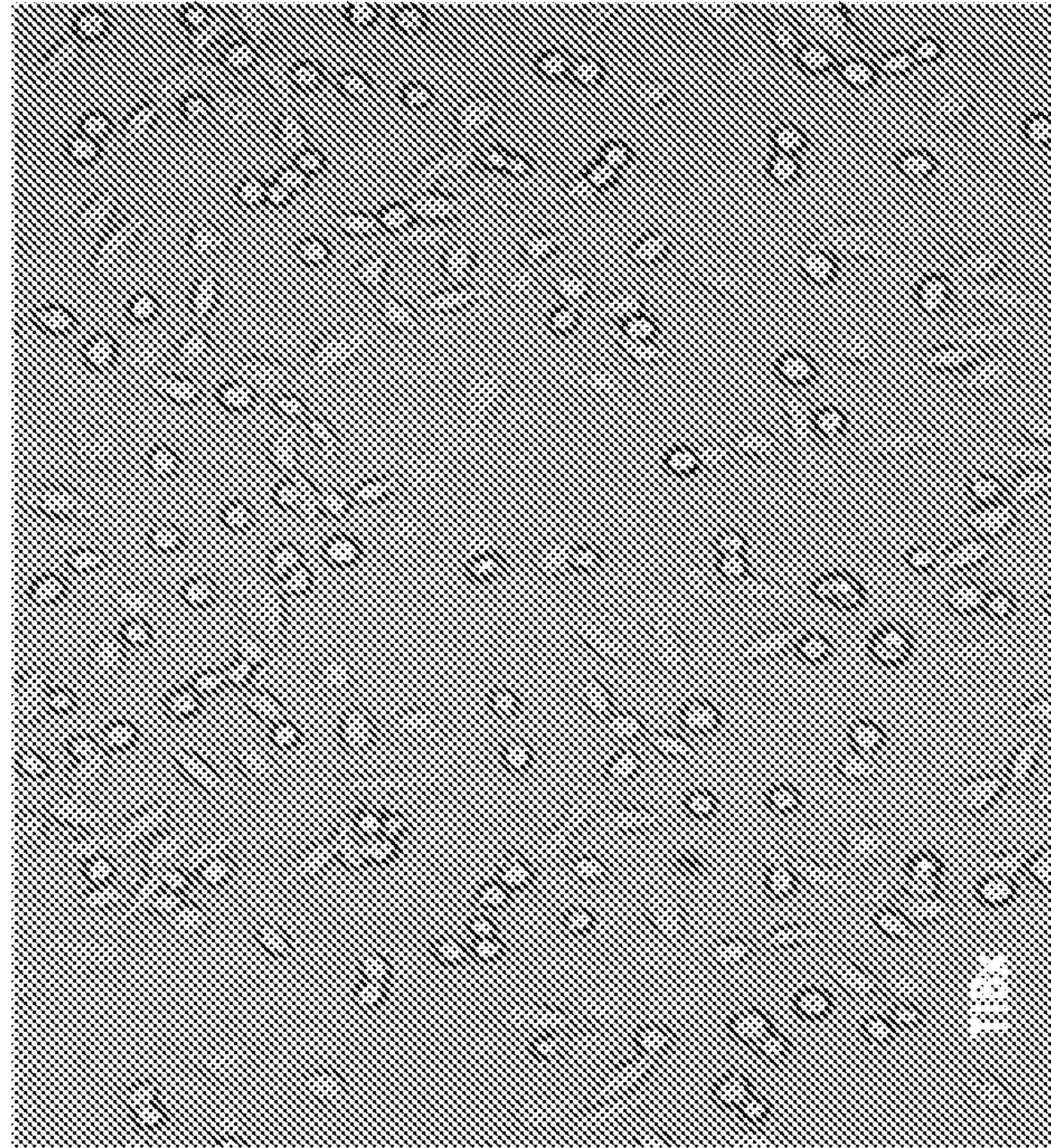
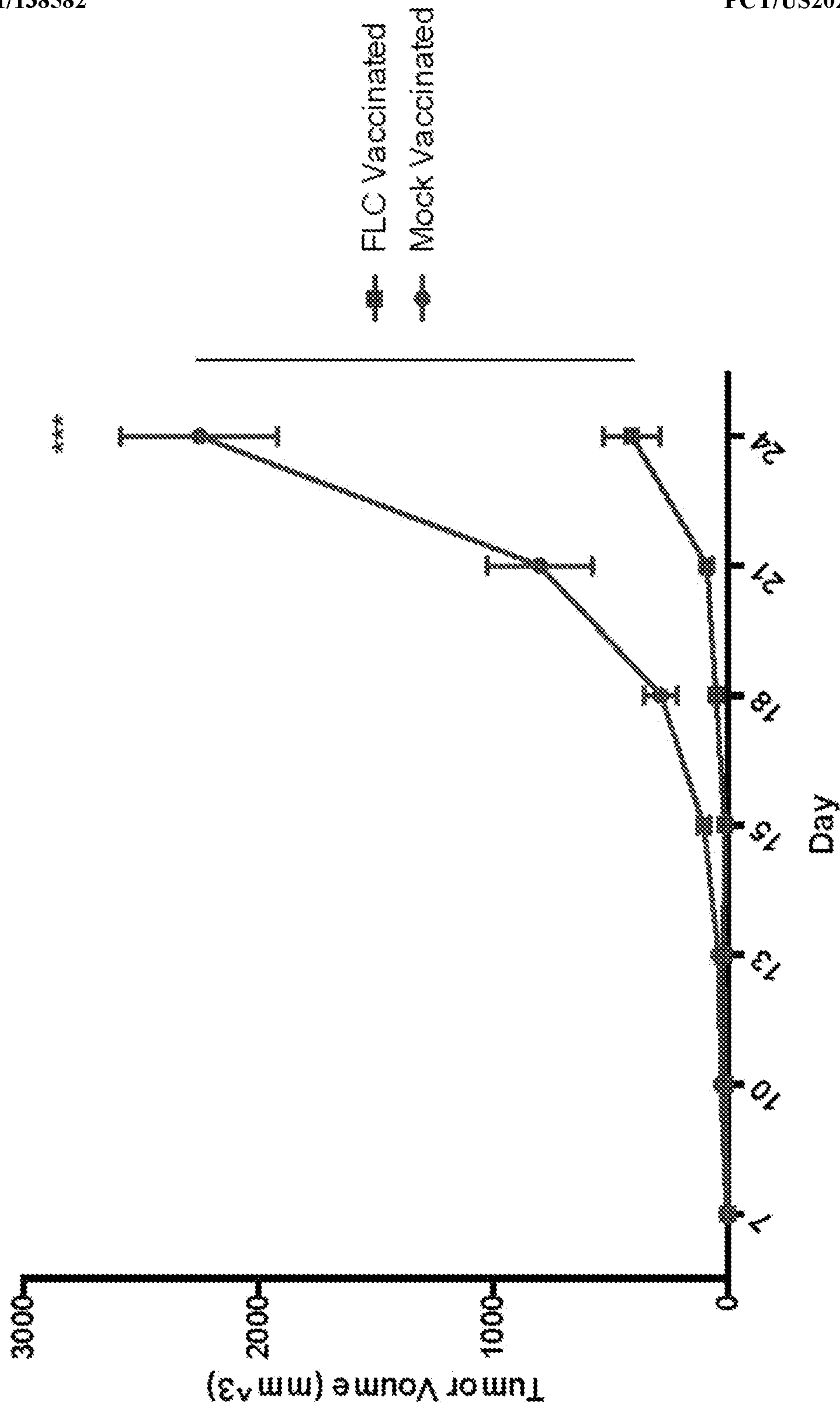


FIG. 4 Tumor Volume With FLC Vaccination Treatment



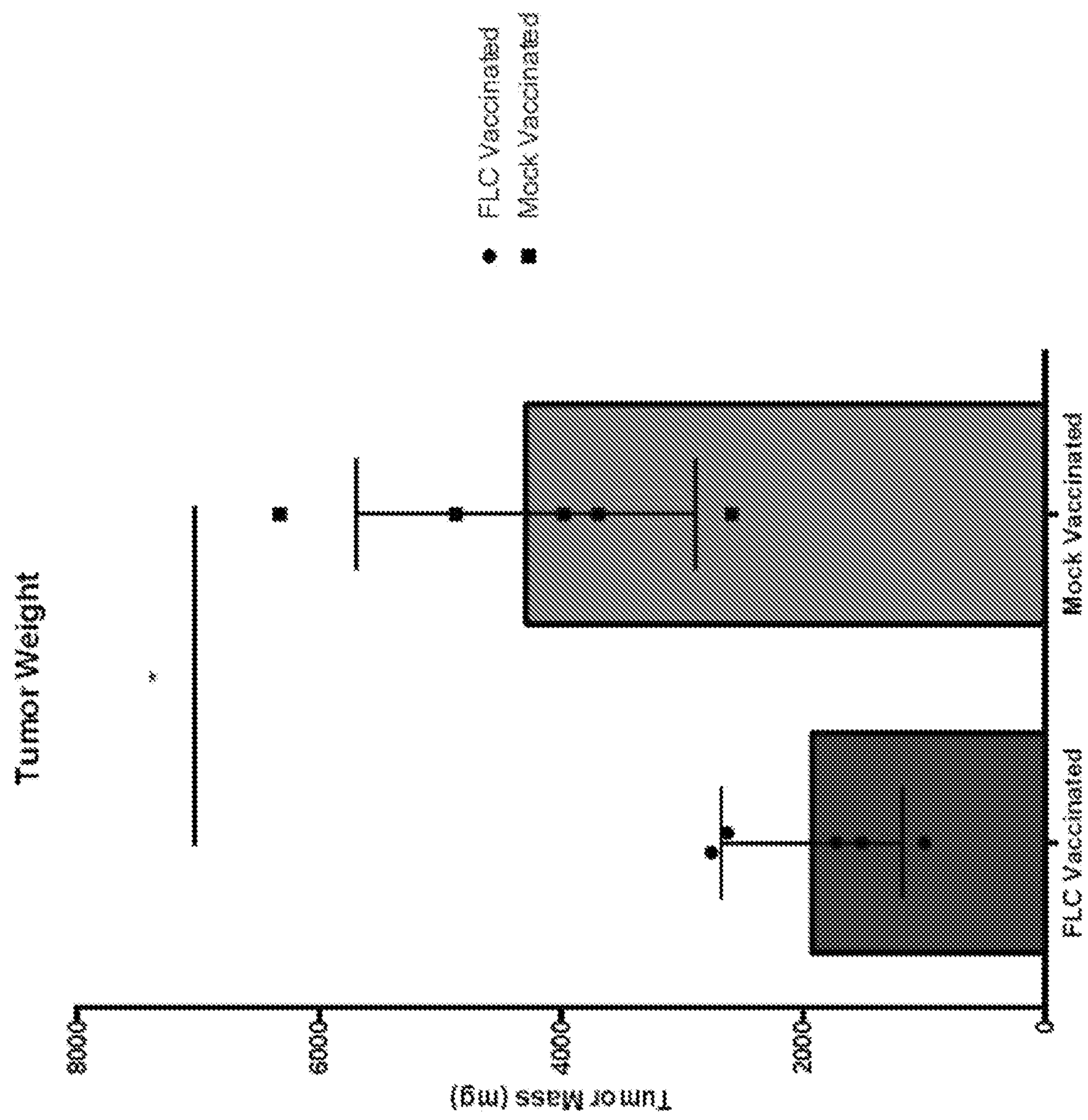


FIG. 5

FIG. 6A

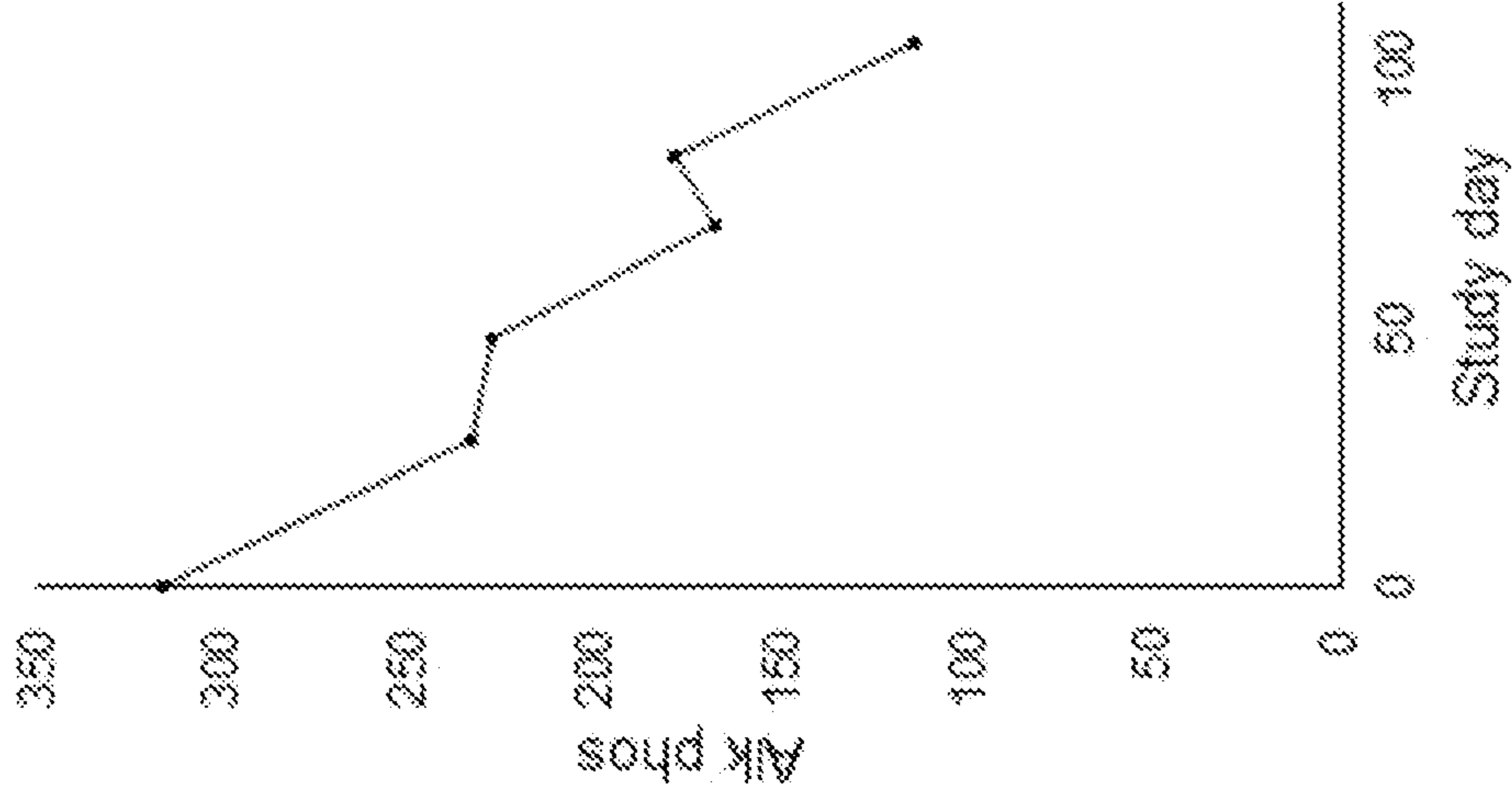
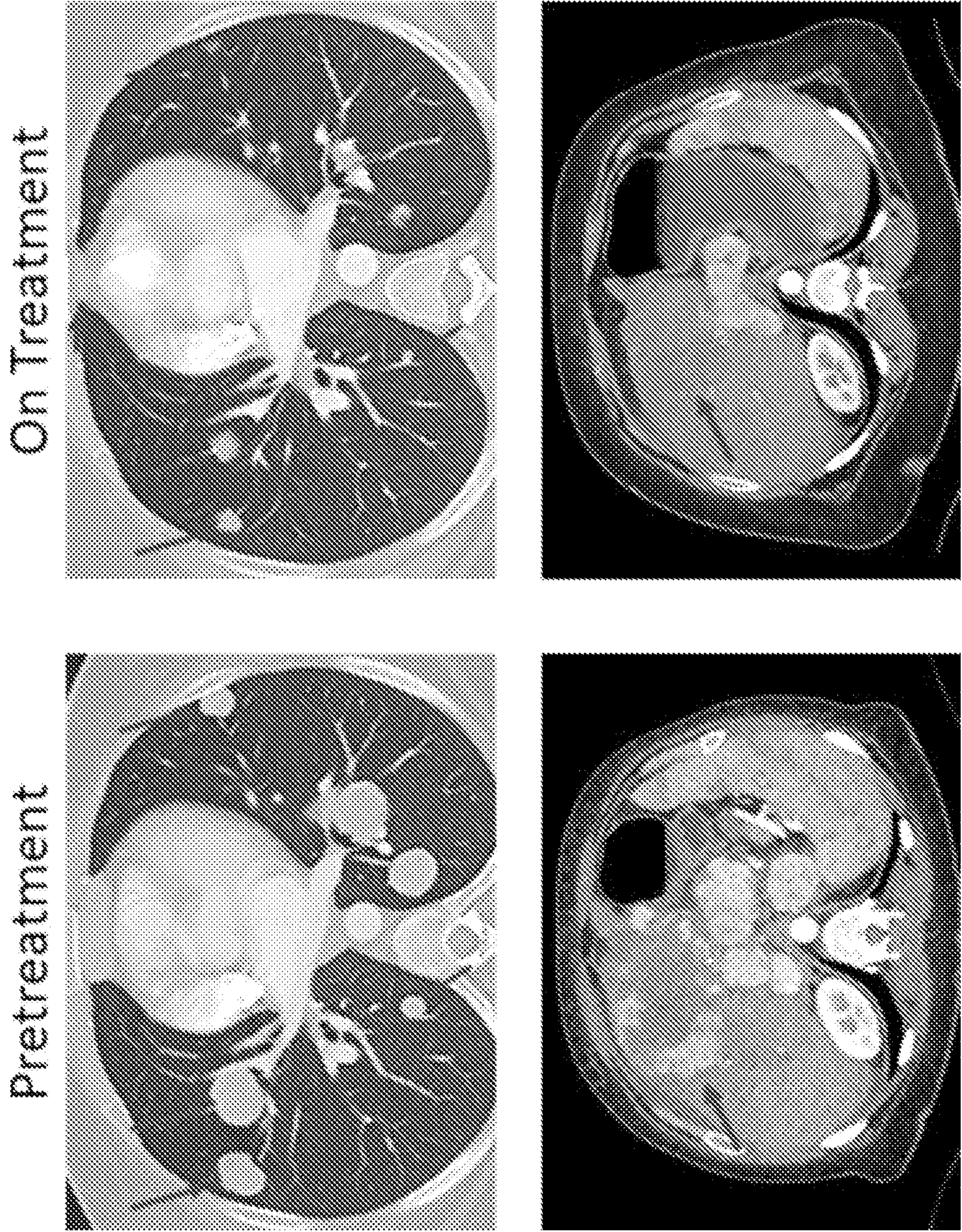


FIG. 6B

FIG. 7

Baseline

Stim	Description	SFU (mean)	SFU (std)
Unstim (dil)	Unstimulated (Negative control)	28.3	28.4
aCD3	anti-CD3 (positive control)	446.7	45.1
CEF	peptide pool (Positive control)	640.0	36.1
Pep1	Investigator peptide {42079_1 IFDRYGEEV}	11.7	7.6
Pep2	Investigator peptide {42079_2 FDRYGEEVK}	11.7	10.4
Pep3	Investigator peptide {42079_3 DRYGEEVKE}	15.0	5.0
Pep4	Investigator peptide {42079_4 RYGEEVKEF}	3.3	2.9
Pep5	Investigator peptide {42079_5 YGEEVKEFL}	8.3	2.9
Pep6	Investigator peptide {42079_6 GEEVKEFLA}	10.0	5.0
Pep7	Investigator peptide {42079_7 EEVKEFLAK}	5.0	5.0
Pep8	Investigator peptide {42079_8 EVKEFLAKA}	13.3	5.8
Pep9	Investigator peptide {40863_1 RKREIFDRYGEEVKEFLAKAKEDF}	13.3	15.3

Week 10 of study therapy

Stim	Description	SFU (mean)	SFU (std)
Unstim (dil)	Unstimulated (Negative control)	18.3	7.6
aCD3	anti-CD3 (positive control)	5.0	0.0
CEF	peptide pool (Positive control)	700.0	45.8
Pep1	Investigator peptide {42079_1 IFDRYGEEV}	26.7	2.9
Pep2	Investigator peptide {42079_2 FDRYGEEVK}	21.7	11.5
Pep3	Investigator peptide {42079_3 DRYGEEVKE}	26.7	2.9
Pep4	Investigator peptide {42079_4 RYGEEVKEF}	15.0	8.7
Pep5	Investigator peptide {42079_5 YGEEVKEFL}	18.3	12.6
Pep6	Investigator peptide {42079_6 GEEVKEFLA}	20.0	5.0
Pep7	Investigator peptide {42079_7 EEVKEFLAK}	36.7	7.6
Pep8	Investigator peptide {42079_8 EVKEFLAKA}	21.7	12.6
Pep9	Investigator peptide {40863_1 RKREIFDRYGEEVKEFLAKAKEDF}	61.7	2.9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/067698

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00; A61P 35/00 (2021.01)

CPC - A61K 39/0011; A61K 39/001196; A61P 35/00; C07K 2319/40 (2021.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2015/048367 A1 (NEW YORK GENOME CENTER, INC. et al) 02 April 2015 (02.04.2015) entire document	1-3, 20-22 ----- 39, 40
X -- Y	US 2017/0029470 A1 (THEVAX GENETICS VACCINE CO., LTD.) 02 February 2017 (02.02.2017) entire document	38 ----- 39, 40
A	WO 2019/090355 A1 (CHILDREN'S NATIONAL MEDICAL CENTER et al) 09 May 2019 (09.05.2019) entire document	1-3, 20-22, 38-40
A	US 2019/0241633 A1 (CUREVAC AG) 08 August 2019 (08.08.2019) entire document	1-3, 20-22, 38-40
A	KASTENHUBER et al. "DNAJB1-PRKACA fusion kinase interacts with β -catenin and the liver regenerative response to drive fibrolamellar hepatocellular carcinoma," Proceedings of the National Academy of Sciences USA, 21 November 2017 (21.11.2017), Vol. 114, No. 50, Pgs. 13076-13084. entire document	1-3, 20-22, 38-40



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

26 March 2021

Date of mailing of the international search report

APR 21 2021

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/067698

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13^{ter}.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13^{ter}.1(a)).

☐ on paper or in the form of an image file (Rule 13^{ter}.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOS: 1-3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, and 47 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/067698

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-19, 23-37, 41-56
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.