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(54) **Title**: T AND B CELL EPITOPES IN SPERM SURFACE PROTEIN SP17 AS ANTI-CANCER VACCINES AND ANTIBODY TARGETS

(57) **Abstract**: Sperm surface protein (Sp17) comprises sequences that are both immunogenic and protective in animal models of human cancers, both in the presence or absence of an adjuvant such as CpG. These Sp17 sequences are further useful for preparing peptides and related pharmaceutical compositions for a variety of therapeutic methods and uses relating to the administration of Sp17-based vaccines or antibody preparations against the occurrence and/or prevention of cancer, in particular of ovarian cancer.

T AND B CELL EPITOPES IN SPERM SURFACE PROTEIN SP17 AS ANTI-CANCER VACCINES AND ANTIBODY TARGETS

[0001] CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Application Serial No. 62/194,882, filed on July 21, 2015, and U.S. Provisional Application Serial No. 62/364,414, filed July 20, 2016; the contents of each are hereby incorporated by reference in their entirety.

[0003] FIELD OF INVENTION

[0004] The present invention relates to peptide antigens corresponding to autoantigenic and therapeutic epitopes present on human sperm surface protein Sp17, in addition to therapeutic and preventive methods employing such peptides and pharmaceutical compositions comprising same, including vaccine and antibody compositions.

[0005] BACKGROUND OF THE INVENTION

[0006] Immunotherapy strategies, including anti-cancer vaccines, are considered to be less toxic and more specific than many current treatments for cancer, including ovarian cancer, and are also regarded as having great potential to significantly benefit cancer patients in need thereof. Several tumor associated antigens have been identified in ovarian cancer cells, including cerebellar degeneration-related protein cdr2, p53, HER2/neu, mesothelin, folate receptor-alpha, cancer testis antigens, such as NY-ESO-1, sperm surface protein Sp17, cancer antigen CA-125 and MUC1 (Kandalaft L. et al., 2011). All of NY-ESO-1, MUC1 and HER-2/neu have been used in clinical trials involving vaccines, with or without other compounds as a combination therapy approach (Diefenbach C. et al., 2008; Loveland B. et al., 2006). However, the lack of meaningful characterization of these antigens, related autoantibodies, and their actual effects on the progression of, and therapies for, ovarian cancers represent factors restricting the number of patients that might be eligible for cancer treatment, although substantial efforts are presently underway attempting to identify the most effective biomarkers that would render such treatments possible. Accordingly, a major limitation in vaccine development against ovarian cancer arises from the absence of well characterized, broadly recognized, crucial antigens expressed by this aggressive form of cancer.

[0007] Sperm surface protein Sp17 (Sp17) comprises 151 amino acids that are highly conserved (i.e. 94% homology between mouse and human), highly expressed in spermatozoa, and presented as a cancer antigen in various models and studies (Arnaboldi F. et al., 2014). Aside from being expressed in the testis, Sp17 is aberrantly expressed in cancers of unrelated histological origin, including multiple myeloma, ovarian cancer, nervous system tumors, and esophageal squamous cell cancer.

[0008] Autoantigens are tissue components of an organism to which that organism directs an immune response, usually of low immunogenicity. However, Sp17 is highly immunogenic *in vivo*, since Sp17-specific cytotoxic T lymphocytes (CTLs) can be easily generated from the peripheral blood of either healthy donors or of cancer patients (Chiriva-Internati M. et al., 2002; Mirandola L. et al., 2015).

[0009] The immunogenicity and high expression on specific cancer cells having restricted expression in normal mammalian tissues make Sp17 an attractive target antigen for cancer immunotherapy strategies. Sp17-derived sequences have shown great potential as candidate vaccines for use in immunotherapy, including the use of adjuvants such as CpG, which was demonstrated in a syngeneic murine model of ovarian cancer where CpG-adjuvant Sp17 vaccines exhibited both therapeutic and prophylactic activity, and also increased overall levels of CTL responses, thereby suggesting that antigen specific responses were induced (Chiriva-Internati M. et al., 2010).

[00010] Although Sp17 acts as an autoantigen in humans, this protein is highly immunogenic *in vivo*. Sp17 protein based vaccines have shown promising protective and therapeutic efficacy in several animal cancer models. However, unnecessary antigenic load in a protein, and production limitations commonly associated with recombinant protein, have hindered vaccine development using the protein target. In contrast, peptide vaccines engineered using short peptide fragments displaying immunogenic epitopes in combination with an optimal carrier/adjuvant, offer an attractive alternative therapeutic strategy, possibly avoiding undesirable anti-allergenic responses. The design of peptide-based vaccines may take advantage of emergent computational paradigms that involve immunoinformatic prediction, thereby facilitating the identification of T cell and B cell epitopes within protein antigens, in particular from cancer (auto) antigens which can be developed into vaccine and antibody targets for a variety of therapeutic uses.

[00011] Sp17-derived peptides have been previously described in connection with autoantigens, for instance, in cancer treatments, diagnosis, and/or vaccination (WO1995/015764; WO2002/068451; WO2013/040071; WO2014/127006). However, these disclosures fail to clearly identify strongly immunogenic, or immunodominant, domains of epitopes within the human Sp17 protein sequence that are responsible for inducing protective immune responses, especially a highly immunogenic epitope region that specifically contains both B and T helper 1 (Th1) cell epitopes. A series of antibodies directed against human and/or mouse Sp17 have been also generated using Sp17-derived sequences and characterized in different experimental models, such as 3C12 (Song J et al., 2014), Clone 22 (Gjerstorff M and Ditzel HJ, 2012), and others (Gupta G et al., 2007; Straughn M et al., 2004), including commercial sources.

[00012] However, a relationship between specific Sp17 epitopes and their efficacy in functionally characterizing candidate vaccines and antibodies has not been clearly established.

[00013] Thus, there is a need for empirically determined Sp17 epitopes and their antibody counterparts necessary to establish the utility of human Sp17 based peptide anti-cancer vaccines and/or antibody-based therapies, in particular as viable therapeutic options against ovarian cancer.

[00014] SUMMARY OF THE INVENTION

[00015] The present invention relates to a fragment of human Sp17 (hSp17) corresponding to amino acids 111-142 (i.e. $hSp17_{111-142}$, having a sequence of KEKEEVAAVKIQAAFRGHIAREEAKKMKTNSL; SEQ ID NO:1), which acts as a strongly immunogenic or immunodominant portion of hSp17. This fragment provides new peptide sequences, each useful as an immunogen, especially for cancer vaccine compositions. The hSp17 fragment is described herein in the context of a therapeutic mouse model, as further characterized using human cells or tissues such as those obtained from clinical samples. This approach further establishes additional peptides and antigenic epitopes that are derived from $hSp17_{111-142}$ useful as anti-cancer vaccines. Moreover, sera raised against such peptides were found to contain high titers of antibody providing not only substantial binding specificity but also substantial cytotoxicity against cancer cells that present Sp17.

[00016] The presently disclosed therapeutic peptides can include 1, 2, 3, or more amino acid substitutions within $hSp17_{111-142}$, preferably conservative amino acid

substitutions, and/or may consist of an $hSp17_{111-142}$ fragment containing from 9 to 31 amino acids. Exemplary peptide sequences include $hSp17_{111-124}$ (corresponding to KEKEEVAAVKIQAA; SEQ ID NO:2), $hSp17_{121-138}$ (corresponding to IQAAFRGHIAREEAKKMK; SEQ ID NO:3), $hSp17_{134-142}$ (corresponding to AKKMKTNSL; SEQ ID NO:4), and $hSp17_{115-133}$ (EVAAVKIQAAFRGHIAREE; SEQ ID NO:5; corresponding to IQ motif of hSp17; Wen et al., 1999). These peptide sequences may be included in a longer peptide that incorporates the sequence of said antigenic fragment, and which further includes from 1 to 10 additional amino acids at the N-terminus and/or the C-terminus.

[00017] The $hSp17_{111-142}$ peptide and the above-mentioned derived sequences (collectively, $Sp17_{epoc}$) are useful as immunogens and in the preparation of pharmaceutical compositions, in particular anti-cancer vaccine compositions, and also in methods for treating or preventing cancer (in particular ovarian cancer), whereby $Sp17_{epoc}$ is preferably administered using a liquid or solid formulation further comprising a carrier, excipients, and/or adjuvants. Accordingly, antibodies directed against $Sp17_{epoc}$ represent useful pharmaceutical compositions, in particular anti-cancer compositions, which are preferably administered using a liquid or solid formulation further comprising a carrier, excipients, and/or adjuvants. These vaccine and antibody compositions are useful in methods for treating or preventing cancer, preferably ovarian cancer,

[00018] A further therapeutic approach relates to methods for generating hSp-17-specific immune effector cells *ex vivo*, comprising pulsing antigen presenting cells with $Sp17_{epoc}$, and contacting the pulsed antigen presenting cells with immune effector cells for a time sufficient to stimulate Sp17-reactive immune effector cells under conditions permissive for proliferation of Sp17-reactive immune effector cells for generating Sp17-specific immune effector cells. The antigen presenting cells can be dendritic cells and the immune effector cells can be cytotoxic T lymphocytes.

[00019] In one aspect, the present invention provides methods of treating cancer by administering to a patient in need thereof a composition comprising a compound based on $Sp17_{epoc}$ that is a $Sp17_{epoc}$ agent, for instance, a $Sp17_{epoc}$-based vaccine or an antibody binding $Sp17_{epoc}$, preferably when $Sp17_{epoc}$ is detected in cancer cells (preferably on the cell surface). In some embodiments, the present invention provides methods of administering $Sp17_{epoc}$ agent together with an agonist therapeutic agent that is directed at one or more adjuvant, one or more anti-cancer agent, or one or more immune effector cells in a subject. Alternatively or in addition, it may be desirable to determine $Sp17_{epoc}$ expression on cancer cells. In some embodiments, $Sp17_{epoc}$

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expression level is determined before, substantially simultaneously with, and/or after administration of one or more doses of a $Sp17_{epoc}$ agent. Those skilled in the art are aware of a variety of techniques for determining such expression levels in a human sample, preferably including one or more biopsies or tumor ascites (as circulating cells, peritumoral cells, and/or intratumoral cells), in particular by determining expression levels using an anti-$Sp17_{epoc}$ antibody according to the present invention.

[00020] In some embodiments, the present methods are applied or administered to subjects that have been diagnosed with cancer, in particular from a cancer that is selected from the group of hematologic malignances including acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia, multiple myeloma, AIDS-related lymphoma, Hodgkin lymphoma, non-Hodgkin lymphoma, Langerhans cell histiocytosis, and myeloproliferative neoplasms. In some embodiments, the cancer is selected from solid tumors including breast carcinoma, squamous cell carcinoma, colon cancer, head and neck cancer, lung cancer, genitourinary cancer, ovarian cancer, rectal cancer, gastric cancer, sarcoma, melanoma, and esophageal cancer.

[00021] The present invention provides certain $Sp17_{epoc}$ agents and related pharmaceutical compositions thereof. In some embodiments, a $Sp17_{epoc}$ agent is or comprises a $Sp17_{epoc}$ antibody, an aptamer, or other non-immunoglobulin compound that specifically binds to $Sp17_{epoc}$. In some embodiments, a $Sp17_{epoc}$ antibody is or comprises an antibody agent that specifically binds to $Sp17_{epoc}$ on surfaces of cancer cells. In some embodiments, the antibody agent is or comprises a polyclonal antibody, a monoclonal antibody, a humanized or human antibody, or includes antigen binding elements of such antibody. In some embodiments, the antibody agent is or comprises $Sp17_{epoc}$ binding elements thereof (i.e. the entire antibody variable regions or selected CDRs sequences within them). In some embodiments, an antibody agent is a multi-specific agent, such as a bispecific antibody, in particular binding specifically to $Sp17_{epoc}$ and to a cancer antigen, including CD19, CD20, CD22, CD38, CD52, CD137, CD33, CD138, CD254, CD261, CD262, CD309, CD319, CD326, PD-1, PD-L1, VEGF, EGFR, and HER3.

[00022] In some embodiments, the $Sp17_{epoc}$ agent may be administered in combination with one or more other agents and/or therapeutic regimens commonly used in the treatment of cancer, in particular those cancer presenting $Sp17_{epoc}$-expressing cells as determined above. Preferably, the $Sp17_{epoc}$ agent and/or therapeutic regimens involve the use and/or administration of chemotherapeutic agents, radiotherapy,

immunotherapeutic agents, and/or inhibitors of kinases and of other cancer-relevant pathways.

[00023] The present invention further provides a variety of kits and/or articles of manufacture containing components relevant to administering $Sp17_{epoc}$ agents for therapeutic uses and/or detection of $Sp17_{epoc}$ expression, particularly the expression within and/or on the surfaces of cancer cells, and within patient samples (such as biopsies, tumor ascites, sera, plasma, and other biological materials).

[00024] Various implementations of the methods and compositions within the scope of the appended claims each have several aspects, no single one of which is solely responsible for the desirable attributes described herein. After considering this discussion, and particularly after reading the sections entitled "Detailed Description" and "Examples" the skilled person will understand how the features of various implementations are used to enable the disclosed $hSP17_{epoc}$–based compositions, suitable for a number of applications, and in particular, for medical use as an anti-cancer therapeutic platform. Examples are provided for purposes of illustration only, and are not intended to limit the scope of the invention. Thus, the invention should be construed to encompass any and all variations to the Examples that become evident as a result of the teachings provided herein.

[00025] **BRIEF DESCRIPTION OF THE DRAWINGS**

[00026] **FIG. 1:** Alignment of human and mouse Sp17 amino acid sequences (Uniprot Acc. No. Q15506 and Q62252, respectively; "=" indicating identical amino acids) together with $hSp17_{1-32}$, $hSp17_{23-54}$, $hSp17_{45-76}$, $hSp17_{67-98}$, $hSp17_{89-120}$, and $hSp17_{111-142}$. Relevant fragments of human Sp17 are underlined; the position of representative sequences comprised in the $Sp17_{epoc}$ definition is also indicated.

[00027] **FIG. 2**: Immunogenicity of recombinant mouse Sp17 (rmSp17) protein combined with a CpG adjuvant in HLA-A2/Kb mice. HLA-A2/Kb mice (n=3/group) were immunized twice (2-weeks apart) with 100 µl of a "rmSp17 (50µg) + CpG (20 µg)" formulation injected at the base of the tail. Mice injected with equal volume of PBS served as controls ("naïve"). 14 days after the last immunization, splenocytes were restimulated with recall antigen peptides in IFN-γ, IL-4 and IL-17 ELISPOT assays; individual mouse samples were tested in triplicate. Data is presented as an average SI (Stimulation Index) of SFU (Spot Forming Unit) ± SD (Standard Deviation; SI=SFU of the antigen (rmSp17) response in vaccinated mice/SFU for the same antigen response

in naïve mice). The dotted line indicates the background level (SI=1); the semi-dotted line indicates the minimal level of a positive response (SI ≥ 2).

[00028] **FIG. 3:** Immunogenicity of hSp17 peptide-based vaccines either combined with a CpG adjuvant or adjuvanted/carried by polystyrene nanoparticles (PSNPs). HLA-A2/K^b mice (n=3-4/group) were immunized twice (intradermally at the base of the tail, 2 weeks apart) with hSp17 peptides ($hSp17_{1-32}$, $hSp17_{23-54}$, $hSp17_{45-76}$, $hSp17_{67-98}$, $hSp17_{89-120}$, and $hSp17_{111-142}$, 50 µg/mouse; see sequences in FIG. 1) combined with a CpG adjuvant (A and B; 20 µg/mouse) or conjugated to PSNPs (C and D; each injection dosage contained ~50 µg peptide and 1% PSNPs). 10-14 days after last immunization, serum was collected from each mouse and assayed for antigen specific antibodies by ELISA. Splenocyte IFN-γ responses to each peptide antigen were measured in triplicate using ELISPOT assays. The Figures each represent 7 different experiments; all data was normalized against responses from the naïve control samples. **FIG. 3A**: IFN-γ responses to CpG-adjuvanted, hSp17-derived peptides. **FIG. 3B**: Antigen specific antibody production (IgG) by CpG-adjuvanted, hSp17-derived peptides. **FIG. 3C**: IFN-γ and IL-17 T cell responses to PSNPs-adjuvanted, hSp17-derived peptides. **FIG. 3D**: Antigen specific antibody production by PSNPs-adjuvanted, hSp17-derived peptides. For FIG. 3A and FIG. 3C, the data is presented as the average SI of SFU ± SD (SI=SFU of the peptide response in vaccinated mice/SFU for the same peptide response in naïve control mice. The dotted lines indicate the background level (SI=1); the semi-dotted lines indicate the minimal level of a positive response (SI ≥ 2). FIG. 3B and FIG. 3D, the data is presented as average SI of OD_{450nm} ± SD (SI = the OD_{450nm} of vaccinated serum/OD_{450} nm of naïve serum at the same dilutions, n = 3–4 individual mice).

[00029] **FIG. 4:** B cell epitope recognition using different adjuvant system. C57BL/6 mice (*n* = 4 mice/group) were immunized 4 times (weekly apart) with $hSp17_{111-142}$-PSNPs vaccine formulation (containing 36 µg /ml of $hSp17_{111-142}$ peptide and 1% PSNPs,

100 µL/mouse/injection) or with CpG-adjuvanted $hSp17_{111-142}$ vaccine formulation (at equivalent peptide dose, *i.e.*, (36 µg peptide + 20 µg CpG)/mouse/injection). Thirteen days after the last immunization, sera were collected, and pooled for each group. **FIG. 4A** and **FIG. 4B**: Two series of peptide fragments within $hSp17_{111-142}$ sequence and $hSp17_{111-142}$ itself were used to compete for the antibody reactivity in sera produced by both vaccine formulations for epitope recognition. **FIG. 4C**: Antibody cross-species reactivity to mSp17 fragments. Five mSp17 peptides were used to compete for antibody

reactivity in sera produced by both vaccine formulations. Data presented as average $OD_{450\ nm}$ ± SD (assayed in triplicate).

[00030] **FIG. 5:** Immunogenicity of formulations wherein $hSp17_{111-142}$ is delivered with CpG or PSNP as an adjuvant in different mice strains. C57BL/6 mice (n=4/group) and HLA-A2Kb mice (n=4/group) were immunized 4 times (intradermally at the base of the tail, 10 days apart) with $hSp17_{111-142}$ peptide (56 µg/mouse) coupled with either CpG (20 µg/mouse) or conjugated to PSNP (1% solid) adjuvants. 8 days after the last immunization, splenocyte IFN-γ responses to the peptide antigen were measured using ELISPOT assays. Serum was collected from each mouse and assayed for antigen specific antibodies by ELISA. **FIG. 5A**: IFN-γ responses. Data is presented as an average SI ± SD (SI=SFU of the peptide response in vaccinated mice/SFU for the same peptide response in naïve mice). **FIG. 5B**: Anti-$hSp17_{111-142}$ specific antibody (IgG) production. ****p<0.0001. **FIG. 5C**: IgG subtypes in C57BL/6 and HLA-A2Kb mice. Data presented as antibody titers ± SD (n= 4 individual mice).

[00031] **FIG. 6:** Comparison of the immunogenicity of rmSP17 protein and $hSp17_{111-142}$ peptide in C57BL/6 mice. C57BL/6 mice (n=3/group) were immunized twice (intradermally, weekly intervals) with rmSp17 protein (100 µg/mouse) or $hSp17_{111-142}$ peptide (100 µg/mouse) combined with CpG (20 µg/mouse) adjuvant. 10 days after the final immunization, splenocyte IFN-γ and IL-17 responses to the immunogen itself were measured in triplicate by ELISPOT assays. Serum from each mouse was collected and assayed for antigen specific antibodies by ELISA. **FIG. 6A**: Antigen specific IFN-γ and IL-17 responses. Data is presented as SI ± SD (SI=SFU of the peptide response/SFU for the same peptide response in naïve control mice). The dotted line indicates the background level (SI=1), and the semi-dotted line indicates the minimal level of a positive response (SI≥2). **FIG. 6B**: Antibody cross-reactivity to rmSp17. Serum from C57BL/6 mice (n=3/group) immunized with $hSp17_{111-142}$ (100 µg/dosage) and rmSp17 protein (100 µg /dosage) both adjuvanted with either CpG (25 µg/dosage) or PSNP (1-%/dosage), were assayed in ELISA coated with rmSp17 protein (5 µg/ml) to test the cross-reactivity of these sera to the rmSp17 protein. Two standard immunizations were carried out intradermally at the base of the tail. Data is presented as an average SI of OD over naïve ± SD (n=3 individual mice).

[00032] **FIG. 7:** Sp17 affects tumor development in mouse models and can be exploited for cancer vaccination and immunotherapy. **FIG. 7A:** Survival curve for C57BL/6 mice (n = 6–8 mice/group) inoculated with murine ovarian cancer (OC) ID8 cells and then treated with CpG-adjuvanted, vaccine formulations containing either

recombinant mouse Sp17 (rmSp17), $hSp17_{111-142}$ peptides, or PBS (control solution); indicating a statistically significant increase of $p < 0.05$ for both treatments compared to PBS treatment (statistical analysis is made by Log-rank. Mantel-Cox test for survival curves). **FIG. 7B:** anti-ID8 lysate specific antibody production in mice groups as established in FIG. 7A indicating a statistically significant increase in both treatment groups CpG-adjuvanted rmSp17 (** $p < 0.01$) and $hSp17_{111-142}$ (*** $p < 0.001$) when compared to PBS treatment (data presented as the average OD_{450} nm ± SD, in triplicate assay). **FIG. 7C:** CFSE staining to compare the growth rates of the $Sp17^+$ and $Sp17^-$ ID8 cells by flow cytometry. Data presented as mean fluorescent intensity (MFI) of CFSE at different time points. **FIG. 7D:** Flow cytometry analysis of Sp17 co-expression with PD-L1 and STAT3 on M4-ID8 cells. Fluorescence due to Sp17 expression is comparatively higher for both STAT3-positive and PDL1-positive M4-ID8 cells when compared to STAT3-negative or to PDL1-negative M4-ID8 cells (data also confirmed in human ovarian cancer cell lines SKOV3 and OVCA433). **FIG. 7E:** Relationship between Sp17 expression & tumorgenicity *in vivo*. Survival curve for tumor induction by Sp17-positive ($Sp17^+$) and Sp17-negative ($Sp17^-$) ID8 cells in a mouse C57BL/6-ID8 model. ID8 cells were inoculated at the dosage of 4 million (4M for both $Sp17^+$ and $Sp17^-$), 8 million (8M, Sp17-negative only) and 10 million cells (10M, Sp17-negative only). **FIG. 7F:** Survival curve comparing the tumorgenicity of ID8 and M4-ID8 ovarian cancer cell lines. C56BL/6 female mice (n=8-10 mice/group) were inoculated with $4x10^6$ original ID8 or M4-ID8 tumor cells via i.p. injection at the lower right flank region.

[00033] **FIG. 8:** Antitumor activity of different anti-hSp17 targeting antibodies and their peptide recognition specificities. Antitumor activities by mouse sera **FIG. 8A** or commercial antibodies **FIG. 8C**. Murine ovarian cancer (OC) cell line M4-ID8 and human OC cell line SKOV3 (5000 cells/well respectively) were treated with both serum (1:200 final dilution) and commercial antibodies (1 µg/ml final) including their corresponding isotype control antibodies and naïve serum. Cell viability was measured by Calcein AM assay 24 hours after treatment. Data is expressed as % of antitumor activity, calculated as % of cell death induced by the serum/antibody treatments (mean ± SD (n=3)). Serum was obtained from mice immunized with either CpG or PSNPs adjuvanted $hSp17_{111-142}$ peptides (two immunizations, 2 weeks apart). Antibody recognition of Sp17 peptide fragments by serum, **FIG. 8B** or commercial antibodies, **FIG. 8D**. Competition studies were made using both serum (1:200 final dilution) and commercial antibodies (1 µg/ml final) with a series of hSp17-derived peptides; peptide-specific antibody recognition is indicated by a reduction in OD_{450nm} readings (EP6496 by

Abcam presents an epitope binding profile similar to N-17 and Proteintech antibodies), its binding being efficiently competed by $hSp17_{1-32}$ $hSp17_{23-54}$ peptides.

[00034] DETAILED DESCRIPTION OF THE INVENTION

[00035] As used herein, the term "administration" refers to the administration of a composition to a subject. Administration to an animal subject, such as a human, can be accomplished via a plurality of routes. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ (for example, intrahepatic, intratumoral, and the like), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal and vitreal. Administration may also involve intermittent dosing. Alternatively, administration may be by continuous dosing (e.g., perfusion) for at least a predetermined period of time. As is known in the art, antibody therapy is commonly administered parenterally, e.g. by intravenous, subcutaneous, or intratumoral injection, for instance, particularly when high doses within a tumor are desired).

[00036] As used herein, the term "agent" may refer to a compound or entity of any chemical class including, for example, polypeptides, nucleic acids, saccharides, small molecules, metals, or combinations thereof. Specific embodiments of agents that may be utilized in accordance with the present invention include small molecules, drugs, antibodies, antibody fragments, aptamers, nucleic acids including but not limited to small interfering RNAs, antisense oligonucleotides, ribozymes, peptides, peptide mimetics, etc. An agent may be or comprise a polymer.

[00037] As used herein, the term "antibody" refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. The skilled person is aware that intact antibodies produced in nature are approximately 150 kD tetrameric agents comprised of two identical heavy chain polypeptides (about 50 kD each) and two identical light chain polypeptides (about 25 kD each) that associate with each other to form a "Y-shaped" structure. Each heavy chain is comprised of at least four domains (each about 110 amino acids long), an amino-terminal variable (VH) domain (located at the tips of the Y structure), followed by three constant domains: CH1, CH2, and the carboxy-terminal CH3 (located at the base of the Y's stem). A short region, known as the "switch", connects the heavy chain variable and constant regions. The "hinge" connects CH2 and CH3 domains to the rest

of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain is comprised of two domains – an amino-terminal variable (VL) domain, followed by a carboxy-terminal constant (CL) domain, separated from one another by another "switch". Intact antibody tetramers are comprised of two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally produced antibodies are also glycosylated, typically on the CH2 domain, and each domain has a structure characterized by an "immunoglobulin fold" formed from two beta sheets (e.g., 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as "complement determining regions" (CDR1, CDR2, and CDR3; as understood in the art, for example determined according to Kabat numbering scheme) and four somewhat invariant "framework" regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen-binding site located at the tip of the Y structure.

[00038] The Fc region of naturally occurring antibodies binds to elements of the complement system, and also to receptors on effector cells, including for example effector cells that mediate cytotoxicity. As is known in the art, affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. The antibodies produced and/or utilized in accordance with the present invention include glycosylated Fc domains; including Fc domains with modified or engineered such glycosylation. For purposes of the present invention, in certain embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an "antibody", whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology.

[00039] In some embodiments, an antibody is polyclonal or oligoclonal, that is generated as a panel of antibodies, each associated to a single antibody sequence and binding a more or less distinct epitopes within an antigen (such as different epitopes within). Polyclonal or oligoclonal antibodies can be provided in a single preparation for

medical uses as described in the literature (Kearns JD et al., 2015). In some embodiments, an antibody is monoclonal. In some embodiments, an antibody has constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. The antibody sequence elements can be humanized, primatized, or chimeric, as known in the art.

[00040] Moreover, the term "antibody" as used herein, can (unless otherwise stated or clear from context) refer to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in an alternative presentation, for instance as antigen-binding fragments, as described below. For example, an antibody utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgG, IgE and IgM, bi- or multi- specific antibodies (e.g., Zybodies®,), single chain variable domains (scFv), polypeptide-Fc fusions, a Fab fragment, a F(ab')2 fragment, a single chain variable fragment (scFv), a scFv-Fc fragment, a single chain antibody (scAb), an aptamer, cameloid antibodies, or a nanobody.

[00041] An antibody or antigen binding fragment thereof, and in particular a monoclonal antibody), may be a rabbit, mouse, chimeric, humanized or fully human antibody or antigen-binding fragment thereof. In some embodiments, a provided antibody or antigen-binding fragment thereof may be of an IgG, IgA, IgE, or IgM isotype, as most suitable for a given use. In some embodiments, a provided antibody or antigen-binding fragment thereof is an IgG isotype, more particularly an IgG1, IgG2, IgG3, or IgG4 isotype, masked antibodies (e.g., Probodies®), or fusion proteins with polypeptides that allow expression and exposure on the cell surface (as scFv within constructs for obtaining artificial T cell receptors that are used to graft the specificity of a monoclonal antibody onto a T cell). In some embodiments, an antibody does not contain a covalent modification, for instance, a glycan attachment that it would have if produced naturally. Alternatively, an antibody may contain a covalent modification, for instance, the attachment of a glycan, a payload such as a detectable moiety, a therapeutic moiety, a catalytic moiety and the like, or other pendant group, for instance, poly-ethylene glycol.

[00042] As used herein, the term "antigen" refers to an agent that elicits an immune response and/or that binds to a T cell receptor, for instance, when presented by an MHC molecule. An antigen eliciting a humoral response involves the production of antigen-specific antibodies as shown in the Examples. For instance, for $Sp17_{111-142}$, a specific $Sp17_{epoc}$ may be used for screening antibody libraries and identifying candidate antibody sequences that can be further characterized.

[00043] As used herein, the term "antigen-binding fragment" refers to an agent that specifically binds to a $Sp17_{epoc}$ peptide, preferably an $Sp17_{111-142}$ sequence. In some embodiments, this term encompasses any polypeptide or polypeptide complex that includes immunoglobulin structural elements sufficient to confer specific binding. Exemplary antigen-binding fragments include, but are not limited to, Small Modular ImmunoPharmaceuticals ("SMIPs™"), single chain antibodies, cameloid antibodies, single domain antibodies (e.g., shark single domain antibodies), single chain or Tandem diabodies (TandAb®), VHHs, Anticalins®, Nanobodies®, minibodies, BiTE®s, ankyrin repeat proteins or DARPINs®, Avimers®, a DART, a TCR-like antibody, Adnectins®, Affilins®, Trans-bodies®, Affibodies®, a TrimerX®, MicroProteins, Centyrins®, CoVX bodies, BiCyclic peptides, Kunitz domain derived antibody constructs, or any other antibody fragments so long as they exhibit the desired antigen binding activity.

[00044] An "antigen-binding fragment" also encompasses alternative protein structures such as stapled peptides, antibody-like binding peptidomimetics, antibody-like binding scaffold proteins, monobodies, and other known non-antibody protein scaffolds (e.g. in Helma J et al., 2015). In preferred embodiments, an antigen-binding fragment is or comprises a polypeptide whose amino acid sequence includes one or more structural elements recognized by those skilled in the art as a complementarity-determining region (CDR). In some embodiments an antigen-binding fragment is or comprises a polypeptide whose amino acid sequence includes at least one CDR (e.g., at least one heavy chain CDR and/or at least one light chain CDR) that is substantially identical to one found in an anti-$Sp17_{epoc}$ sequence; amino acid sequence, and in particular in anti-$Sp17_{epoc}$--HCDR3 sequence. In some embodiments, an included CDR is substantially identical to an anti-$Sp17_{111-142}$ amino acid sequence, and in particular to the anti-$Sp17_{111-142}$-HCDR3 sequence, in that it is either identical in sequence or contains 1, 2, 3, 4, or more amino acid substitutions that do not change its binding or biological activity.

[00045] As used herein, the terms "biological sample" or" "sample" typically refers to a sample obtained or derived from a biological source of interest, for instance, a tissue or organism or cell culture. One source of interest can be an animal or human organism. The biological sample may comprise one or more biological tissues or fluids.

[00046] As used herein, the terms "cancer", "tumor", and "carcinoma", are used interchangeably herein to refer to cells that exhibit relatively abnormal, uncontrolled, and/or autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. In general, cells of

interest for detection or treatment in the present application include precancerous (benign), malignant, pre-metastatic, metastatic, and non-metastatic cells. The present disclosure may be applicable to any and all cancers. Non-limiting examples include hematopoietic cancers such as leukemias, lymphomas (Hodgkins and non-Hodgkins), myelomas and myeloproliferative disorders; sarcomas, melanomas, adenomas, carcinomas of solid tissue, squamous cell carcinomas of the mouth, throat, larynx, and lung, liver cancer, genitourinary cancers such as prostate, cervical, bladder, uterine, ovarian, and endometrial cancer and renal cell carcinomas, bone cancer, pancreatic cancer, skin cancer, cutaneous or intraocular melanoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, head and neck cancers, breast cancer, gastro-intestinal cancers and nervous system cancers, and benign lesions such as papillomas.

[00047] As used herein, the term "combination therapy" refers to those situations in which a subject is simultaneously exposed to two or more therapeutic regimens, such as exposure to two or more therapeutic agents. In some embodiments, two or more agents may be administered simultaneously. Alternatively, such agents may be administered sequentially; otherwise, such agents are administered in overlapping dosing regimens.

[00048] As used herein, the term "comparable" refers to two or more agents, entities, situations, effects, sets of conditions, and the like that may not be identical to one another but that are sufficiently similar to permit comparison between them such that conclusions may reasonably be drawn based on differences or similarities observed. Such comparable sets of conditions, effects, circumstances, individuals, or populations are characterized by a plurality of substantially identical features and one or a small number of varied features. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, effects, or populations, and the like are considered comparable.

[00049] A composition or method described herein as "comprising" one or more named elements or steps is open-ended, meaning that the named elements or steps are essential, but other elements or steps may be added within the scope of the composition or method. It is also understood that any composition or method described as "comprising" (or which "comprises") one or more named elements or steps also describes the corresponding, more limited composition or method "consisting essentially of" (or which "consists essentially of") the same named elements or steps,

meaning that the composition or method includes the named essential elements or steps and may also include additional elements or steps that do not materially affect the basic and novel characteristic(s) of the composition or method.

[00050] As used herein, the term "dosage form" refers to a physically discrete unit of an active agent including a therapeutic or diagnostic agent, for administration to a subject. Each unit contains a predetermined quantity of active agent. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (i.e., with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or agent administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

[00051] As used herein, the term "dosing regimen" refers to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length. Alternatively, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. Alternatively, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. A dosing regimen may comprise a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount and it is correlated with a desired or beneficial outcome when administered across a relevant population, namely a therapeutic dosing regimen.

[00052] As used herein, the term "epitope" refers to a portion of an antigen that is bound by an antibody. In some embodiments, where the antigen is a polypeptide, an epitope is conformational in that it is comprised of portions of an antigen that are not covalently contiguous in the antigen but that are near to one another in three-dimensional space when the antigen is in a relevant conformation. For example, for $Sp17_{epoc}$ agent, conformational epitopes are those comprised of amino acid residues

that are not contiguous in $Sp17_{111-142}$ sequence; linear epitopes are those comprised of amino acid residues that are contiguous in $Sp17_{111-142}$ sequence.

[00053] As used herein, the term "patient" or "subject" refers to any organism to which a provided composition is or may be administered, for example, for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals including but not limited to mammals such as mice, rats, rabbits, non-human primates, and/or humans. In some preferred embodiments, a patient is a human. In some embodiments, a patient is suffering from or susceptible to one or more disorders or conditions. A patient may display one or more symptoms of a disorder or condition, or may have been diagnosed with one or more disorders or conditions (such as cancer, or presence of one or more tumors). In some embodiments, the patient is receiving or has received certain therapy to diagnose and/or to treat such disease, disorder, or condition.

[00054] As used herein, the term "pharmaceutically acceptable" applied to the carrier, diluent, or excipient used to formulate a composition as disclosed herein means that the carrier, diluent, or excipient must be compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

[00055] As used herein, the term "pharmaceutical composition" refers to a composition in which an active agent is formulated together with one or more pharmaceutically acceptable carriers. A pharmaceutical compositions may be formulated for administration in solid or liquid form, including those adapted for oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, for example, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous, intradermal, intratumoral, or epidural injection as a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to skin, lungs, or oral cavity; intravaginally, intrarectally, sublingually, ocularly, transdermally, nasally, pulmonary, and to other mucosal surfaces.

[00056] As used herein, the term "solid tumor" refers to an abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumors may be benign or malignant. Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors are sarcomas (including cancers arising from transformed cells of mesenchymal origin in tissues such as cancellous bone, cartilage, fat, muscle,

vascular, hematopoietic, or fibrous connective tissues, carcinomas (including tumors arising from epithelial cells), melanomas, lymphomas, mesothelioma, neuroblastoma, retinoblastoma, and the like.

[00057] As used herein, the term "therapeutically effective amount" refers to an amount, for instance, of an agent or of a pharmaceutical composition, that is sufficient when administered to a population suffering from or susceptible to a disease and/or condition in accordance with a therapeutic dosing regimen for treating such disease and/or condition. A therapeutically effective amount is one that reduces the incidence and/or severity of, stabilizes, and/or delays onset of, one or more symptoms of the disease, disorder, and/or condition. Those of ordinary skill in the art will appreciate that a "therapeutically effective amount" does not in fact require successful treatment be achieved in a particular subject.

[00058] As used herein, the term "treatment" (also "treat" or "treating") refers to any administration of an $Sp17_{epoc}$ agent (e.g., $Sp17_{epoc}$-based vaccine or anti- $Sp17_{epoc}$ antibody) that partially or completely alleviates, ameliorates, relives, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms.

[00059] Agents that are exemplary anti-$Sp17_{epoc}$ antibodies according to the present invention providing clinical relevance to cancer therapy can be generated using technology that are commonly applied for producing monoclonal antibodies and other protein-binding agents. For example, mice can be immunized with a human $Sp17_{epoc}$ ($hSp17_{epoc}$, such as $Sp17_{111-142,}$ with or without the use of an adjuvant such CpG or nanoparticles, as described in the Examples) to generate panels of B cells suitable for cell fusion using the mouse splenocytes and a mouse myeloma cell line, after confirming the presence of $hSp17_{epoc}$ immunoreactivity in the serum of immunized mice. Alternatively, human sera can be screened for identifying human subjects already presenting such $hSp17_{epoc}$ immunoreactivity and human B cell samples can be isolated from such human subjects for generating hybridomas using known techniques. The corresponding clonal cells and hybridomas can then be used for screening clones expressing monoclonal antibodies that bind to peptides representing $hSp17_{epoc}$ (or fragments thereof) and/or for cancer relevant functional activities, such as the lysis of mouse and/or human cancer cell clones (as shown in the Examples with ovarian cancer cell lines).

[00060] In some embodiments, binding to $hSp17_{epoc}$ peptide can be determined by screening the culture supernatants derived from hybridoma cells using an antibody

capture ELISA directly against human and murine $Sp17_{epoc}$ peptides. In other embodiments positive clones can be also screened for binding to the entire recombinant human Sp17 protein and/or for binding to cells that are stained within human primary and metastatic tumors and tissues (for example, from ovarian cancer samples compared to normal ovarian samples) and/or in a wide panel of commonly studied human cancer cell lines (for ovarian cancer SKOV3, ID8, OVCA433) by cytochemistry or by flow cytometry, using as control Sp17-negative cell lines (as shown in the examples, in murine Sp17-positive and Sp17-negative ID8 cell lines). Anti-$Sp17_{epoc}$ body binding activity (avidity and affinity) can be further quantified using standardized analytical methods, such as methods based on Biocore assay, using peptides corresponding to $Sp17_{epoc}$ that are covalently linked to the surface of a Sensor Chip CM5 via primary amine coupling. A sample containing the candidate anti-$hSp17_{epoc}$ monoclonal antibody is contacted with the receptor surface to establish binding on-rate and off-rate properties and binding affinities. Libraries of antigen-dinging sequence and or other protein libraries (such as phage display libraries) can be similarly screened.

[00061] In some embodiments, positive outcomes from such initial binding screening can be further validated, after verifying monoclonality, in a secondary screen for binding and biological interaction with cancer cells (such as ovarian cancer primary cells or ovarian derived cell lines) and/or the absence of binding or other biological activity for other epitopes present in hSp17, in other proteins or other cells (such as normal tissues). The Examples disclose several representative approaches involving the use of sera and antibodies for determining the lytic, cytotoxic activity on Sp17-positive and Sp17-negative ID8 cell lines in addition to other cell lines.

[00062] The protein sequence within such anti-$hSp17_{epoc}$ candidates can then be cloned in the most appropriate protein scaffold (for instance, immunoglobulin-derived or non-immunoglobulin derived) for evaluating the additional features that can make such agent most suitable for therapeutic use, for example, cytotoxicity, stability, biodistribution, recombinant production in cell lines for pharmaceutical uses, lack of aggregation, means for purification, and the like.

[00063] For example, in one embodiment, the one or more candidate anti-$hSp17_{epoc}$ monoclonal antibodies can be characterized for their complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) against human or mouse cancer cell lines (such as human SKOV3 ovarian cancer cell line). When compared to a control antibody (such as isotype control antibody or an antibody for unrelated antigen or Sp17 epitope), the candidate one or more anti-$hSp17_{epoc}$

monoclonal antibodies present a dose-response cytotoxicity over a range of concentrations such as 0.01, 0.1, 1 and 10 µg/ml concentration, using fresh normal human serum (NHS; for CDC assay) or freshly isolated PBMCs from healthy donor (for ADCC assay) as the source of complement or effector cells respectively. After this co-culture step, the lactase dehydrogenase (LDH) released in culture media upon cell lysis can be measured following a colorimetric reaction. The amount of color formed is proportional to the number of lysed cells.

[00064] In some embodiments, the one or more candidate anti-hSp17$_{epoc}$ monoclonal antibodies can be further evaluated for a direct therapeutic activity in immunocompetent or immunodeficient animal cancer models, for instance, as described in the Examples with respect to ovarian cancer, in which cancer cell lines are inoculated intravenously or intraperitoneally. The properties of the one or more candidate anti-hSp17$_{epoc}$ monoclonal antibodies can be compared against other therapies, such as conventional first-line chemotherapeulic agents, like carboplatin and paclitaxel, or other antibodies binding antigens present on ovarian cancer cells.

[00065] In some embodiments, outcome criteria including the development of ovarian cancer, weight, immune effector and therapeutic functions (ADCC, CDC and potential to enhance immunity through the use in combination with checkpoint inhibitors targeting the same cells), tumor ascites formation, or other clinical score can be regularly monitored in each group of control and treated mice (with or without sacrificing them) over one or more months. Additionally, the one or more candidate anti-hSp17$_{epoc}$ monoclonal antibodies can be tested alone or in combination with suitable anti-cancer drugs for evaluating additional valuable effects, for example, synergism, decreasing negative side effects or drug resistance, reducing the effective dose or the period of treatment, reducing metastasis or recurrence, increasing the percentage of responders to the therapy. The formulation of the one or more candidate anti-hSp17$_{epoc}$ monoclonal antibodies with the anti-cancer drug can be the same (e.g. within the same liquid solution) or share the same carrier (e.g. nanoparticles loaded with both drugs). Overall viability and cancer recurrence can provide (together with pharmaceutical criteria such as biodistribution and safety) a pre-clinical evaluation of the one or more candidate anti-hSp17$_{epoc}$ monoclonal antibodies for a potential therapeutic use prior to human testing.

[00066] In some embodiments, following selection for their hSp17$_{epoc}$ binding capacity and/or antitumor activity, the one or more candidate anti-hSp17$_{epoc}$ monoclonal antibodies can be characterized by sequencing the variable regions characterizing each antibody and identifying the relevant CDR sequences. The entire antibody variable

sequences (or the isolated CDRs from heavy and light chains) can be recloned within the (no-)immunoglobulin protein scaffold most appropriate in terms of recombinant production, stability during storage, pharmacokinetics, immunological properties, tissue penetration, conjugation with (radio)chemicals, size or other criteria that are relevant for the final medical use of resulting protein (either for therapeutic or diagnostic uses). In some embodiments, the one or more candidate anti-hSp17$_{epoc}$ monoclonal antibody can be also comprised in a single, multispecific (e.g. bispecific) construct together with other antigen-binding sequences that bind other cancer targets including checkpoint inhibitors, or antigen characterizing cancer cells of general or specific subtypes (e.g. PD-1, PD-L1, CTLA-4, LAG3, TIM3, CD28, CD20, EGFR, CD52, OX40, CD137, HER2 and the like; for a review, see Redman J et al., 2015). In some embodiments, a provided antibody or antigen-binding fragment thereof is included in an agent that further comprises a conjugated payload such as a therapeutic or diagnostic agent. In such embodiments, the agent is considered and/or referred to as an "immunoconjugate".

[00067] In some embodiments, the present invention provides anti-hSp17$_{epoc}$ sequences that identify antibodies or antigen-binding fragments thereof. In some embodiments, such sequences identify antibodies or antigen-binding fragments thereof that bind an epitope in hSp17$_{epoc}$, and optionally, also a corresponding epitope of Cynomologous monkey and/or murine Sp17$_{epoc}$,, either as isolated proteins or on the surface of cells expressing hSp17$_{epoc}$ (such as ovarian cancer cells or other cancer cells and cell lines).

[00068] The present invention also provides nucleic acid molecules encoding an isolated antibody or antigen-binding fragment thereof comprising anti Sp17$_{epoc}$, amino acid sequences. In some embodiments, such nucleic acid molecules may contain codon-optimized nucleic acid sequences, and/or may be included in expression cassettes within appropriate nucleic acid vectors for the expression in host cells such as, for example, bacterial, yeast, insect, murine, simian, or human cells. Such host cells may comprise heterologous nucleic acid molecules (such as DNA vectors) that express the antibody or antigen-binding fragment thereof (comprising anti Sp17$_{epoc}$ amino acid sequences).

[00069] The present disclosure further provides methods of preparing an isolated antibody or antigen-binding fragment thereof as described herein, for instance, comprising anti-Sp17$_{epoc}$ amino acid sequences. Such methods may comprise culturing a host cell that comprises nucleic acids (e.g., heterologous nucleic acids that may comprise and/or be delivered to the host cell via vectors). The host cells (and/or the

heterologous nucleic acid sequences) can be arranged and constructed so that antibody or antigen-binding fragment thereof is secreted from host cells such that the antibody can be isolated from cell culture supernatants and/or exposed on the cell surface (for instance, if such anti-$Sp17_{epoc}$ amino acid sequences are intended to be used in the context of, or together with, such cells, e.g. artificial T cell receptors grafting an antibody specificity onto T cells).

[00070] Pharmaceutical compositions comprising an $Sp17_{epoc}$ agent for use according to the present invention may be prepared for storage and/or delivery using any of a variety of techniques and/or technologies known to those skilled in the art. In particular, the $Sp17_{epoc}$ agent is administered according to a dosing regimen approved by a regulatory authority such as the United States Food and Drug Administration (FDA) and/or the European Medicines Agency (EMEA), e.g., for a given indication. In some embodiments, use of $Sp17_{epoc}$ agent may permit reduced dosing, for example, a lower amount of active compound in one or more doses, a smaller number of doses, and/or a reduced frequency of doses) of an approved agent used in combination with the $Sp17_{epoc}$ agent.

[00071] In some embodiments, dosing and administration of the $Sp17_{epoc}$ agent utilizes an active agent having a desired degree of purity combined with one or more physiologically acceptable carriers, excipients or stabilizers in any or variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions including injectable and infusible solutions, dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. In some embodiments, a preferred form may depend on the intended mode of administration and/or therapeutic application, typically in the form of injectable or infusible solutions, such as compositions similar to those used for treating of human subjects with vaccines or antibodies.

[00072] In some embodiments, ingredient(s) of the composition according to the invention can be prepared with carriers that protect the agent(s) against rapid release and/or degradation, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as polyanhydrides, polyglycolic acid, polyorthoesters, and polylactic acid. In general, each active agent is formulated, dosed, and administered in therapeutically effective amount using pharmaceutical compositions and dosing regimens that are consistently with good medical practice and appropriate for the relevant agent(s) including antibody agents. Pharmaceutical compositions containing active agents according to the invention can be administered by any

appropriate method known in the art, including, without limitation, oral, mucosal, by-inhalation, topical, buccal, nasal, rectal, or parenteral, for instance intravenous, infusion, intratumoral, intranodal, subcutaneous, intraperitoneal, intramuscular, intradermal, transdermal, or other modes of administration involving a physical breach of a tissue of a subject and subsequent administration of pharmaceutical composition through such breach.

[00073] In some embodiments, a dosing regimen for a $Sp17_{epoc}$ agent may involve intermittent or continuous (e.g. by perfusion or slow release system) administration, for example to achieve a particular desired pharmacokinetic profile or other pattern of exposure in one or more tissues or fluids of interest in the subject. In some embodiments, different agents administered in combination may be administered via different routes of delivery and/or according to different schedules. Factors to be considered when optimizing routes and/or dosing schedule for a given therapeutic regimen may include, for example, the particular cancer being treated (such as type, stage, location), the clinical condition of a subject (including age, overall health, weight), the site of delivery of the agent, the nature of the agent (e.g. an antibody or other protein-based compound), the mode and/or route of administration of the agent, the presence or absence of combination therapy, and other factors familiar to medical practitioners.

[00074] Those skilled in the art will appreciate that a specific route of delivery may impact the required dose amount. For example, where particularly high concentrations of an agent within a particular site or location (within an tissue or organ) are of interest, focused delivery (such as intratumoral delivery) may be desired and/or useful. In some embodiments, one or more features of a particular pharmaceutical composition and/or of a utilized dosing regimen may be modified over time (increasing or decreasing amount of active in any individual dose, increasing or decreasing time intervals between doses, etc.), for instance, in order to optimize a desired therapeutic effect or response (e.g., a therapeutic or biological response that is related to $Sp17_{epoc}$ expression on cell surface).

[00075] In general, the type, amount, and frequency of dosing of active agents in accordance with the present invention in governed by safety and efficacy requirements that apply when relevant agent(s) is/are administered to a mammal, preferably a human. In general, such features of dosing are selected to provide a particular, and typically detectable, therapeutic response as compared with what is observed absent therapy. In context of the present invention, an exemplary desirable therapeutic response may

involve, but is not limited to, inhibition of and/or decreased tumor growth, tumor size, metastasis, one or more of the symptoms and side effects that are associated with the tumor, as well as increased apoptosis of cancer cells, therapeutically relevant decrease or increase of one or more cell marker or circulating markers and the like. Such criteria can be readily assessed by any of a variety of immunological, cytological, and other methods that are disclosed in the literature. For example, the therapeutically effective amount of $Sp17_{epoc}$ agent alone or in combination with a further agent, can be determined as being sufficient to kill cancer cells.

[00076] A therapeutically effective amount of an active agent or composition comprising same can be readily determined using techniques available in the art including, for example, considering one or more factors such as the disease or condition being treated, the stage of the disease, the age and health and physical condition of the mammal being treated, the severity of the disease, other previous or ongoing treatments, and the like. In some embodiments, a therapeutically effective amount is an effective dose (and/or a unit dose) of an active agent that may be at least about 0.01 µg/kg body weight, at least about 0.05 µg/kg body weight; at least about 0.1 µg/kg body weight, at least about 1 µg/kg body weight, at least about 5 µg/kg body weight, at least about 10 µg/kg body weight, or more (e.g. about 100 µg/kg body weight). It will be understood by one of skill in the art that such guidelines may be adjusted for the molecular weight of the active agent. The dosage may also be varied for route of administration, the cycle of treatment, or consequently to dose escalation protocol that can be used to determine the maximum tolerated dose and dose limiting toxicity (if any) in connection to the administration of the $Sp17_{epoc}$ agent at increasing doses.

[00077] Therapeutic compositions should typically be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and other required ingredients from those enumerated above. In the case of powders for preparing sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile filtered solution. The proper fluidity of a solution can be maintained, for example,

by using a coating, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[00078] The formulation of the $Sp17_{epoc}$ agent according to the invention is preferably sterile, as accomplished by filtration through sterile filtration membranes, and then packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations as discussed herein. Sterile injectable formulations may be prepared using a non-toxic parenterally acceptable diluent or solvent, such as water or 1,3 butanediol. Other parenterally administrable formulations that are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer or salt.

[00079] The pharmaceutical composition for use in accordance with the present invention may include pharmaceutically acceptable dispersing agents, wetting agents, suspending agents, isotonic agents, coatings, antibacterial and antifungal agents, carriers, excipients, salts, or stabilizers are non-toxic to the subjects at the dosages and concentrations employed. A non-exhaustive list of such additional pharmaceutically acceptable compounds includes buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; salts containing pharmacologically acceptable anions (such as acetate, benzoate, bicarbonate, bisulfate, isothionate, lactate, lactobionate, laurate, malate, maleate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, thiethiodode, and valerate salts); preservatives (such as octadecyidimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; sodium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine,

glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

[00080] In some embodiments, where two or more active agents are utilized in accordance with the present invention, such agents can be administered simultaneously or sequentially. In some embodiments, administration of one agent is specifically timed relative to administration of another agent. In some embodiments, desired relative dosing regimens for agents administered in combination may be assessed or determined empirically, for example using *ex vivo, in vivo* and/or *in vitro* models; in some embodiments, such assessment or empirical determination is made *in vivo*, in a particular patient or patient population (such that a correlation is made). The $Sp17_{epoc}$ agent and one or more active agents utilized in practice of the present invention are administered according to an intermittent dosing regimen comprising at least two cycles. Where two or more agents are administered in combination, and each by such an intermittent, cycling, regimen, individual doses of different agents may be interdigitated with one another. One or more doses of the second agent is administered a period of time after (or before) a dose of $Sp17_{epoc}$ agent.

[00081] In another embodiment of the invention, the $Sp17_{epoc}$ agent is provided as a container with a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition that is effective for treating the condition and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). For example, the formulation is packaged in clear glass vials with a rubber stopper and an aluminum seal. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. Such container can be included in an article of manufacture that may further comprise a separate container comprising a pharmaceutically acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint. For example, the article of manufacture may allow providing each or the agent in an intravenous formulation as a sterile aqueous solution containing a total of 2 mg, 5 mg, 10 mg, 20 mg, 50 mg, or more that are formulated, with

appropriate diluents and buffers, at a final concentration of 0.1 mg/ml, 1 mg/ml, 10 mg/ml, or at an higher concentration.

[00082] The $Sp17_{epoc}$ agent (especially in the form of a vaccine or anti-$Sp17_{epoc}$ antibody) can be provided within the kits-of-parts in the form of lyophilized is to be reconstituted with any appropriate aqueous solution that provided or not with the kits, or other types of dosage unit using any compatible pharmaceutical carrier. One or more unit dosage forms of the isolated antibody or antigen-binding fragment thereof comprising the $Sp17_{epoc}$ agent may be provided in a pack or dispenser device. Such a pack or device may, for example, comprise metal or plastic foil, such as a blister pack. In order to use correctly such kits-of-parts, it may further comprise buffers, diluents, filters, needles, syringes, and package inserts with instructions for use in the treatment of cancer. The instructions that are associated to the article of manufacture and/or the kits of the invention may be in the form of a label, a leaflet, a publication, a recording, a diagram, or any other means that can be used to inform about the correct use and/or monitoring of the possible effects of the $Sp17_{epoc}$ agents, formulations, and other materials in the article of manufacture and/or in the kit.

[00083] The present invention is illustrated by means of the following non-limiting Examples.

[00084] **EXAMPLES**

[00085] **EXAMPLE 1: Characterization of Sp17 Epitopes and Anti-hSp17 Antibodies relevant for Cancer Vaccination**

[00086] **Materials & Methods**

[00087] *Recombinant Sp17 protein and peptides*

[00088] Mouse recombinant Sp17 protein (rmSp17) was provided by Dr. Chiriva-Internati (Texas Tech University, USA). Six 32-mer long human Sp17 overlapping peptides ($hSp17_{1-32}$, $hSp17_{23-54}$, $hSp17_{67-98}$, $hSp17_{89-120}$, $hSp17_{45-76}$ and $hSp17_{111-142}$) were designed (FIG. 1) and commercially synthesized by e.g. GenScript (NJ, USA) or Mimotopes (Clayton, Australia). Using an online epitope prediction program (http://www.syfpeithi.de/), 23 of predicted 8-, 9-mer CD8 epitopes, having high binding affinity to the HLA-A2.1 and H-2Kb and H-2Db molecules across both human and mouse Sp17 amino acid sequence, and 7 of B cell epitopes in C-termini of human Sp17 region ($hSp17_{111-142}$) (Tables 1 and 2) were designed and synthesized.

[00089] *Vaccine formulations*

[00090] rmSp17 protein and six of the overlapping peptides from the human Sp17 protein sequence, namely $hSp17_{1-32}$, $hSp17_{23-54}$, $hSp17C_{45-76}$, $hSp17_{67-98}$, $hSp17_{89-120}$ and $hSp17_{111-142}$, were used as vaccine antigens. Each of the individual peptides were either mixed with CpG (ODN 1826, Invivogen, USA) directly or conjugated to 40-50 nm carboxylated polystyrene nanoparticles (PSNPs, Polysciences Inc., Warrington, USA). Each vaccine antigen was mixed with the indicated amount of CpG in PBS prior to injection.

[00091] Peptide conjugation to PSNPs was optimized for each peptide in order to achieve the best conjugation efficiency and size (40–60 nm), following the conjugation procedures as described previously (Xiang S. et al., 2013), using two conjugation buffer systems (MES and PBS) and a range of pH conditions (pH 5.5 to pH 8) that were tested for each peptide conjugated to PSNPs. In brief, when using MES (2-N-Morpholino-ethanesulfonic acid), PSNPs at a final concentration of 1% solids were pre-activated in a mixture containing MES (50 mM final, pH=6), 1-ethyl-3-(3-dimethylaminopropryl) carbodiimide hydrochloride (EDC; 4 mg/mL final; Sigma-Aldrich, St. Louis, USA), N-hydrosulfosuccinimide (Sulfo-NHS; 50 mM final; Pierce™, Thermo Fisher Scientific, Waltham, Massachusetts, USA), and adjusted to a pH of 5.5–6.5. After preactivation, the excess activation agents (EDC and Sulfo-NHS) were removed from the preactivation mix using a gel filtration column (Zeba spin desalting column following manufacturer instructions; ThermoFisher Scientific); the buffer was exchanged at the same time via the column (with optimized buffer conditions for each antigen), before adding the peptide antigen for a further 2 hours. The final conjugation mix was then dialyzed against PBS in 1 kDa dialysis membrane. All hSp17 peptides were successfully conjugated to the PSNPs in an optimal size range (< 60 nm) and efficiency (% peptide effectively conjugated to the PSNPs ≥ 60%). Final conjugation efficiency was determined by BCA™ protein assay (Pierce™ Micro BCA protein assay, Thermo Fisher Scientific) and sizes were measured by Zetasizer (Malvern Instruments Ltd, Worcestershire, UK).

[00092] Each vaccine dose (~100 µl) contained 50-100 µg peptides with 20 µg CpG or ~1% solid of PSNPs in PBS. The amounts of peptide antigen injected were matched for both formulations by adjusting the injection volume for each experiment.

[00093] *Immunizations, immunogenicity and immune-therapy*

[00094] To study the immunogenicity of the Sp17 protein and peptide antigens, 6-8 week old female C57BL/6 (H-2Kb) and HLA-A2.1 [A2KbC57BL/6JTgN(A2KbH2b)6Hsd)]

transgenic mice sourced from Monash Animal Services (MAS) and Animal Resources Centre (ARC, Western Australia) were used. Mice were immunized with 100 µl of each vaccine formulation at various concentrations intradermally at the base of the tail. Details of each immunization schedule are set forth in the description and comments to the respective Figure herein. Injections with PBS alone served as a negative control ("naïve"). For multiple immunizations, mice were typically boosted with the same formulation 7-10 days apart (see description and comments to the respective Figure for each experiment). 10-14 days following the final immunization, mice were euthanized by CO_2 asphyxiation, their spleens removed and splenocytes harvested for subsequent immunogenicity assays (ELISPOT). Serum was also collected from each mouse prior to immunization and at cull for detecting antigen specific antibodies using ELISA.

[00095] *ELISPOT and ELISA assays*

[00096] Antigen specific CD4, CD8 or Th17 T cell responses were evaluated by IL-4, IFN-γ and IL-17 ELISPOT assays. Briefly, 96-well filtration plates (MSIP or MAIP plates, Millipore, Billerica, MA) were coated with 100 µl/well of either anti-mouse IFN-γ (AN18, 5 µg/ml, MABTech, Stockholm, Sweden), anti-mouse IL-17 (5 µg/ml, MABTech) or rat anti-mouse IL-4 (5 µg/ml, BD Pharmingen, San Diego, USA). Following overnight incubation at 4°C, the wells were washed and blocked with RPMI 1640 completed medium (CM) supplemented with 10% heat inactivated fetal calf serum (Australian origin, supplied by Invitrogen), 2 mM glutamine, 100 µg/ml streptomycin and 100 units/ml penicillin, 0.2 mM β-mercaptoethanol and 20 mM Hepes (all from Gibco, Life Technologies, California, USA), Splenocytes (50 µl) taken from immunized mice (2 x 10^7 cells/ml, either individual or pooled) were added to triplicate wells and incubated with 50 µl of recall antigens (rmSp17 protein or Sp17 peptides) at various concentrations (2.5 - 25 µg/ml for all potential CD8 epitopes and 25 - 100 µg/ml for long peptides and protein) in a 37°C incubator filled with 5% CO_2 for a minimum of 16 hours for IFN-γ plates, 24 hours for IL-4 plates and 36-40 hours for IL-17 plates. Concanavalin A (Con-A) (1 µg/ml final, Amersham Biosciences, Uppsala, Sweden) was used as a positive control and CM was added to background wells. The plates were then washed 6 times in PBS and incubated with 100 µl biotinylated detection antibodies [anti-mouse IFN-γ biotinylated mAb R4-6A2 (Mabtech); anti-mouse IL-17 biotin (Mabtech); rat anti-mouse IL-4 biotin (BD), all at 1 µg/ml] at room temperature for 1-2 hours. After washing as above, steptavidin-alkaline phosphatase (to detect IFN-γ and IL-17, MabTech) or Extravidin-ALP (for detection of IL-4, Sigma-Aldrich) was added (final at 1 µg/ml) and

incubated for another 1.5 hours at room temperature. Plates were then washed again, with a final wash using Reverse Osmosis (RO) water to remove residual PBS. The spots were developed using a colorimetric AP kit (Bio-Rad, Philadelphia, USA) following the manufacturers' instructions. Spots were counted using an AID ELISPOT Reader System (Autoimmun Diagnostika GmbH, Germany). The magnitude of the specific cytokine induction in response to the recall antigen were compared either directly for its spot forming unit (SFU) or normalized against the corresponding naïve response to the same recall antigen, calculated as the stimulation index (SI) of SFU over naïve (SI = [SFU from the treatment mice] / [SFU from the naïve mice] for each corresponding recall antigen). An antigen-specific response was considered to be positive only when the SI ≥ 2 and net SFU ≥ 20 per million cells.

[00097] Serum samples were collected from vaccinated animals prior to immunization and when euthanized; each sample was assayed for antigen-specific antibody production by ELISA. Briefly, 96-well plates were coated with rmSp17 protein or peptides diluted in carbonate/bicarbonate coating buffer (5 µg/ml, 50 µl/well) and incubated overnight at 4°C. After washing with PBS/0.05% Tween-20 and blocking with 5% skim milk, serial dilutions of mouse sera were added and incubated at 37°C for 2 hours or 4°C overnight. After washing as above, HRP-conjugated sheep anti-mouse IgG (Amersham) was added and allowed to incubate at 37°C for another 1 hour. The reaction was developed using TMB substrate (Invitrogen, USA) and stopped with 1 M HCl, before reading the absorbance at 450 nm (OD_{450nm}). The magnitude of the antibody levels were compared either directly by its OD_{450nm} reading or normalized against the corresponding naïve serum at the same dilution point (also calculated as SI = [OD_{450nm} for test serum] / [OD_{450nm} for naïve serum] at the same dilution point). Antibody endpoint titers represent the degree to which the serum could be diluted and still contain detectable amounts of antibody, and were calculated as the serum dilution at which the OD_{450nm} was equal to the mean OD of the serum of naïve mice + 3 standard deviations (SD).

[00098] Competition ELISA were performed by incubating the test serum (usually at 1:100 - 1:400 dilutions, depending on the antibody titers) with competing peptides (serially diluted from 10 µg/well) at 1:1 ratio (volume:volume) in a 96-well tissue culture plate for 1 hour in a 37°C incubator. Following incubation, 50 µl of competition mix from each well were transferred to corresponding ELISA plates that were pre-coated (and blocked) with the same competing peptide. Standard ELISA protocol was carried out as

described above. Changes in OD_{450nm} value reflect cross-reaction between antibody and competing peptides.

[00099] *Statistical analysis*

[000100] Statistical analysis was performed using one-way or two-way ANOVA using Graph Pad Prism v6.04 software and Excel. Differences were considered statistically significant at $p < 0.05$. Group sizes are indicated in the description and comments to the Figure. The values are expressed as mean ± SD or as average.

[000101] **Results**

[000102] *Sp17 has an immunogenic region containing both B cell and Th1 cell epitopes*

[000103] To progress the rational development of Sp17 peptide based vaccines, the T cell and B cell epitope regions in this protein that are immunogenic and potentially the target of protective immunity, as well as antibodies with specificity for relevant human Sp17 sequences were identified. Identifying epitopes that are relevant in immunocompetent murine models is highly significant in this context. The model currently available to test how natural Sp17 expression can be the target of protective immunity is based on the murine ID8 cell line, derived from a spontaneous ovarian carcinoma arisen in C57BL/6 mice. To map potential CD8 T cell epitopes in Sp17 in this strain, we thus synthesized predicted H-2Kb and H-2Db binding peptide epitopes. For future vaccine development in humans, it was also of interest to determine potential HLA-A2.1 (the most common human MHC class I type) binding CD8 T cell epitopes.

[000104] In order to map the CD8 T cell epitopes in the Sp17 peptide as well as identify the immunogenic regions in human Sp17 as a potential vaccine target, we utilized an epitope prediction program (SYFPEITHI; http://www.syfpeithi.de/) to predict potential CD8 epitopes. A significant number of high affinity HLA-A2.1 and H-2Kb epitopes clustering at both N-terminal 1-76 amino acid and C-terminal 111-142 amino acid regions were predicted.

[000105] We therefore designed six overlapping 32-mer long human Sp17 peptides, namely $hSp17_{1-32}$, $hSp17_{23-54}$, $hSp17_{45-76}$ $hSp17_{67-98}$, $hSp17_{89-120}$,and $hSp17_{111-142}$ as potential peptide vaccine targets (see FIG. 1). We also synthesized 10 predicted high affinity H2-Kb and HLA-A2.1 binding CD8 T cell epitopes in regions identical between mouse and human Sp17, and another 7 of HLA-A2.1 and 6 of H-2Kb restricted CD8 T cell epitopes peptides, respectively, in the remaining human and mouse Sp17 sequence

(predominantly C-terminal) as 8-mer and 9-mer peptides. The six overlapping peptides were assayed for their ability to elicit CD8 T cells from C57BL/6 or HLA-A2.1 transgenic mice. Mice were immunized twice intradermally with $hSp17_{1-32}$, $hSp17_{23-54}$, $hSp17_{45-76}$, $hSp17_{67-98}$, $hSp17_{89-120}$, or $hSp17_{111-142}$ peptides with CpG adjuvant, as well as recombinant murine Sp17 protein (rmSp17). In the positive control formulation (rmSp17+CpG), despite positive IFN-γ responses being detected upon stimulation with rmSp17 protein by IFN-γ ELISPOT assay (FIG. 2), no reactivity could be detected to any of the minimal MHC Class I binding predicted peptides in either mouse strain (Table 1) at multiple concentrations (range from 2.5-25 μg/ml). Similarly, for the CpG adjuvanted hSp17 peptides based formulations, no reactivity could be detected to any of the minimal MHC class I binding predicted peptides (13 peptides total) in HLA-A2Kb mouse strain (Table 2) at multiple concentrations (range from 2.5-25 μg/ml).

[000106] **TABLE 1: Screening for potential CD8 T cell epitopes in rmSp17 protein***

Position	Sequence	Sequence homology between human and mouse	rmSp17 reactivity
2-10	SIPFSNTHY	100%	-
11-19	RIPQGFGNL	100%	-
12-19	IPQGFGNL	100%	-
12-20	IPQGFGNLL	100%	-
18-26	NLLEGLTRE	100%	-
19-27	LLEGLTREI	100%	-
22-30	GLTREILRE	100%	-
27-35	ILREQPDNI	100%	-
30-38	EQPDNIPAF	100%	-
34-42	NIPAFAAAY	100%	-
38-46	FAAAYFESL	89%	-
39-46	AAAYFENL	89%	-
39-47	AAAYFESLL	89%	-
40-47	AAYFENLL	89%	-
45-53	SLLEKREKT	89%	-
66-74	DRFYNNHAF	100%	-
91-99	QISGKEEET	11%	-
111-119	KEKEEVAAV	56%§	-

115-123	AALKIQSLF	56%[§]	-
116-124	VAAVKIQAA	56%[§]	-
134-142	AKKMKTNSL	44%[§]	-
136-143	KSDKNENL	22%	-
136-144	KSDKNENLK	22%	-
rmSp17	recombinant murine Sp17 protein		++++[ξ]

[*]To screen potential CD8 T cell epitopes in rmSp17, both C57BL/6 mice and HLA-A2K[b] transgenic mice (n=3-4/group) were immunized twice with CpG (20 µg/mouse) adjuvanted rmSp17 protein (50 µg/mouse) vaccine formulations intradermally, 2 weeks apart. 10-14 days after last immunization, splenocytes were tested for reactivity in triplicate to each of the 23 predicted CD8 T cell epitopes in IFN-γ ELISPOT assays. TABLE 1 shows summary results from 4 independent experiments. A positive response was determined by the stimulation index (SI) of the SFU of recall peptide in vaccinated mouse/SFU for the same peptide in naïve mouse. The SI ≥ 2 considered as a positive response. [§]: the homology was adjusted between mouse and human because of the identical amino acid shift in those regions. [ξ]: indicated strong positive responses with SI>10.

[000107] **TABLE 2: Screening for potential CD8 T cell epitopes in hSp17 peptides#**

Position	Sequence	IMMUNOGEN			
		$hSp17_{1-32}$	$hSp17_{23-54}$	$hSp17_{45-76}$	$hSp17_{111-142}$
2-10	SIPFSNTHY	-			
11-19	RIPQGFGNL	-			
12-20	IPQGFGNLL	-			
18-26	NLLEGLTRE	-			
19-27	LLEGLTREI	-			
22-30	GLTREILRE	-	-		
27-35	ILREQPDNI		-		
34-42	NIPAFAAAY		-		
45-53	SLLEKREKT			-	
91-99	QISGKEEET[&]				
111-119	KEKEEVAAV[@]				-
116-124	VAAVKIQAA[@]				-
134-142	AKKMKTNSL				-

To screen potential CD8 T cell epitopes in the human Sp17 overlapping peptides, HLA-A2Kb transgenic mice (n=3-4/group) were immunized twice with CpG (20 µg/mouse) adjuvanted hSp17 peptides ($hSp17_{1-32}$, $hSp17_{23-54}$, $hSp17_{45-76}$, $hSp17_{67-98}$, $hSp17_{89-120}$, and $hSp17_{111-142}$, 50 µg/mouse) vaccine formulations intradermally, 2 weeks apart. 10-14 days after last immunization, splenocytes

were tested in triplicate for reactivity to each of the 13 predicted HLA-A2.1 restricted CD8 T cell epitopes in IFN-γELISPOT assays. & no binding to $hSp17_{67-98}$. @ no binding to $hSp17_{89-120}$. TABLE 2 shows summary results from 5 independent experiments. A positive response was determined by the stimulation index (SI) of the SFU of recall peptide in vaccinated mouse/SFU for the same peptide in naïve mouse. A SI ≥ 2 was considered as a positive response.

[000108] However, if low levels of IFN-γ production were induced by $hSp17_{45-76}$ peptide to itself (and only when using PSNPs as adjuvant), substantial IFN-γ T cell responses, and high antibody titers are observed only when using $hSp17_{111-142}$ peptide to itself, with none of the other five hSp17 peptides-PSNPs formulations inducing any antigen specific antibody responses. No detectable IL-17 or IL-4 T cell responses were induced by any of the peptide regions, including the full peptide $hSp17_{111-142}$ (FIG. 3 and other data not shown). Collectively these results confirm, using two distinguishable adjuvant systems, that the $hSp17_{111–142}$ peptide comprises a primary immunogenic region within hSp17 useful for vaccine and antibody development.

[000109] Since the above results show that the peptide conjugation process may have exposed new useful B cell epitopes in $hSp17_{111–142}$, we proceeded to map the B cell epitopes within $hSp17_{111–142}$ using a panel of small peptide fragments within the $hSp17_{111–142}$ (FIG. 4A and FIG. 4B). Such peptides were intended to block the recognition by the anti-$hSp17_{111–142}$ antibody that is induced by $hSp17_{111–142}$ formulation in a competition ELISA assay. Surprisingly, only some peptide fragments including $hSp17_{121-138}$ (for CpG-adjuvanted $hSp17_{111–142}$ vaccine formulation) and $hSp17_{131-142}$ or $hSp17_{134-142}$ (for PSNPs-adjuvanted, hSp17-derived peptides) demonstrated competing activity for antibody binding. These findings suggest narrowing down the identified B cell epitope to a sequence region of 139–142 amino acids (TNSL). Since "TNSL" corresponds to the C-terminal portion of the $hSp17_{111–142}$ peptide sequence, the conjugation of the peptide to PSNPs could potentially destroy the epitope or at least change the epitope accessibility. The change in immunogenicity by 121–138 amino acids, in turn, may have occurred by promoting exposure of a region otherwise masked by the rest of the peptide. Notably, none of the murine equivalent peptides could efficiently block the binding to $hSp17_{111–142}$ by antibodies induced by either $hSp17_{111–142}$ formulation (FIG. 4C).

[000110] To further investigate the suitability of the $hSp17_{111-142}$ as a vaccine target, the immunogenicity of the $hSp17_{111-142}$ was also examined in different mice strains. C57BL/6 mice and HLA-$A2K^b$ transgenic mice were immunized with the $hSp17_{111-142}$ peptide based formulations with CpG or PSNP adjuvants. The overall magnitude of the IFN-γ response induced by the $hSp17_{111-142}$ peptide was significantly higher in C56BL/6

mice than in HLA-A2.1 mice for the same formulation tested, and the response to the CpG adjuvant formulation was higher than that to the PSNP adjuvant formulation in both mice strains (FIG. 5A). On the contrary, for antibody responses, both formulations induced similar levels of IgG in HLA-A2.1 mice, which were also comparable to the CpG adjuvanted formulation in C57BL/6 mice. But, to a large extent, the PSNP adjuvant formulation induced much lower antibody responses in C57BL/6 mice (FIG. 5B). The use of a PSNP based delivery platform for the $hSp17_{111-142}$ peptide also resulted in a different antibody IgG subtype being induced compared to the CpG adjuvant formulation. The CpG adjuvant formulation predominantly induced IgG2a and IgG2b, whereas the PSNP-conjugated formulation induced IgG1 in C57BL/6 mice. The pattern was slightly different in HLA-A2.1 mice, with the CpG adjuvant formulation inducing IgG2a, and the PSNPs-conjugated formulation inducing both IgG2a and IgG1 subtypes (FIG. 5C).

[000111] Full length rmSp17 protein with CpG adjuvant has been shown to be immunoprotective, providing therapeutic efficacy after nine repeated immunizations in C57BL/6 mice. Efficacy was suggested in these studies to be mediated by either CD8+ T cells or Th17 cells. In the present Examples, there was no evidence for CD8 T cell or Th17 cell induction after 2 immunizations with any of the overlapping hSp17 peptides (Table 2). However, surprisingly strong Th1 and antibody responses were detected to the $hSp17_{111-142}$ peptide. To further validate the potential usage for the $hSp17_{111-142}$ peptide as a vaccine target and to be able to utilize the murine ID8 OC tumor model in future studies, we compared the $hSp17_{111-142}$ with the rmSp17 in the induction of immunogenicity in C57BL/6 mice when both antigens were coupled with a CpG adjuvant. FIG. 6 shows that both formulations induced Th1, but not Th17 antigen-specific responses to the immunizing antigen (FIG. 6A). Furthermore, serum from $hSp17_{111-142}$ peptide based vaccines (CpG or PSNP adjuvant formulations) showed different levels of cross-reactivity to the rmSp17 protein (coating with the rmSp17 protein in ELISA; FIG. 6B).

[000112] Collectively these results demonstrate that formulating immunogenic peptides into nanoparticle based vaccine platforms are well-suited to alter the specificity, the nature (antibody isotype), and the cross-reactivity of the generated antibody response, compared to a standard pro-inflammatory mixed with adjuvants, such as CpG. Specifically, the present inventors have shown that although it is possible to generate functional antibody responses to an immunogenic fragment of hSp17 (i.e. the reference $hSp17_{111-142}$ peptide, or the fragments and variants that were tested in this Example 1, and as further defined as $hSp17_{epoc}$), using such hSp17 fragments in combination with

one or more noninflammatory PSNPs as a vaccine delivery system, promotes a qualitatively different response to CpG, particularly in the nature of the antibody response elicited. Consequently, this approach also is particularly advantageous for establishing panels of B cells to be used for generating hybridomas and selecting monoclonal antibodies that present the desired specificity for $hSp17_{epoc}$ peptides and isotype.

[000113] **EXAMPLE 2: Cancer-specific Activities of Compounds Targeting hSp17**

[000114] **Materials & Methods**

[000115] *Cell lines and Cell Preparations*

[000116] The mouse ovarian MOSEC cell line clone ID8, more commonly known as ID8 cells, were kindly provided by Dr. Katherine Roby (University of Kansas Medical Center, USA; Roby K. et al., 2000). The ID8 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) or RPMI supplemented with 4% FBS and 1x ITS (Insulin-Transferrin-Selenium; Mediatech/Corning). ID8 cells were selected on the basis of Sp17 expression by limiting dilution method, and the single cell clones were tested for Sp17 expression by flow cytometry. The $Sp17^+$ and Sp17-negative ID8 clones were expanded in cell culture for generating cell preparations of $Sp17^+$ and Sp17-negative ID8 clones. Mice were inoculated with $Sp17^+$ ID8 clones cell preparations, tumor mass was then isolated from each diseased mouse, and cultured further *in vitro* before reinoculated in another group of mice. Again, tumor cells were isolated from the tumor mass, and cultured *in vitro*. This new ID8-originated cell line was designated "M4-ID8", and characterized as a highly tumorigenic variant of ID8 M4-ID8 cells..

[000117] *Therapeutic effect of hSp17-based vaccines*

[000118] The ID8 murine ovarian cancer model was first established in C57BL/6 mice. Briefly, $4x10^6$ ID8 murine OC cells in PBS (100 µl) were inoculated intraperitoneally at the low right flank (abdomen) area. Three weeks after the initial inoculation, mice were treated with various vaccine formulations injected intradermally at the base of the tail. Each treatment group was repeated in triplicate with the same formulation 3 weeks apart. Mice were monitored daily for the first appearance of a tumor, and measured daily for weight and abdominal circumference. Survival was followed until the circumference reached 100 mm (due to the build-up of ascites fluid), or until killed earlier due to complications arising from the tumor. Serum antibody were collected at the day of cull for each mouse, pooled and assayed (at 1:100 dilution) for antigen

specific reactivity to ID8 cell lysate. Each vaccine dose (~100 µl) contained 50-100 µg peptides with 20 µg CpG or ~1% solid of PSNPs in PBS were prepared and administered as described in Example 1.

[000119] *Analysis of Sp17-positive and Sp17-negative ID8 cells.*

[000120] In the cell proliferation assay using Carboxyfluorescein succinimidyl ester (CFSE a fluorescent cell staining dye that is used to monitor cell proliferation), equal amounts ($4x10^6$ cells) of Sp17-positive and Sp17-negative ID8 cells were washed and were stained with CFSE (0.4 µM final) for 5 minutes in 37°C incubator. CFSE staining was quenched by addition of cold cell culture media, followed by washing in PBS. The pre-stained cells were then cultured in complete medium. Samples of such cells were harvested at different time points and fixed in 1% PFA prior to flow cytometry analysis.

[000121] Flow cytometry analysis of Sp17 co-expression with PDL1 and STAT3 was performed using $5x10^5$ M4-ID8 cells stained with PE labeled anti-PDL1 (rat anti-mouse CD274; BD Biosciences, cat. No. 558091) and Alexa Fluor® 647 labeled mouse anti-STAT3 (pY705; BD Biosciences, cat. No. 557815), or indirectly stained using mouse anti-Sp17 (A-12; SantaCruz Cat. No. sc-365325) followed by FITC-conjugated secondary antibody. Controls included labeled isotypes and secondary antibody alone. After staining and washing, cells were fixed in 1% PFA. Flow cytometry data was acquired with BD™ LSR II Cell Analyzer (BD Biosciences), and analyzed using Flowjo analysis software (TreeStar).

[000122] *Model for ovarian cancer based on Sp17-positive and Sp17-negative ID8 cells*

[000123] In the model comparing the tumorigenicity of Sp17-positive and Sp17-negative ID8 cells, the different ID8 cell preparations were inoculated to 6 weeks old C57BL/6 female mice (n=5 mice/group) via intraperitoneal injection in the indicated amount. Tumor formation and growth were monitored over a period of 153 days in all groups, with mice in Sp17-negative ID8 cell groups all surviving at the end of the experiment (143 days, and culled at day 153, except Sp17- ID8/8M cells group which were monitored till day 254 before cull).

[000124] In the model comparing the tumorgenicity of ID8 cells and M4-ID8 cells, $4x10^6$ cells of each cell preparations were inoculated in C56BL/6 female mice (n=8-10 mice/group) via intraperitoneal injection at the lower right flank region. Tumor formation and growth were monitored over a period of 126 days. Survival was followed until the

mouse abdominal circumference reached 100 mm (due to the build-up of ascites fluid) or killed earlier if any complications occurred due to the tumor progression.

[000125] *Anti-sp17 agents*

[000126] The $hSp17_{111-142}$–specific sera were produced using mice immunized with either CpG- or PSNPs-adjuvanted $hSp17_{111-142}$ peptide (each mouse received two immunizations, administered 2 weeks apart) and tested at 1:200 final dilution. The commercial anti-human Sp17 agents that were tested at 1µg/ml final concentration were: goat anti-Sp17 (N-17; SantaCruz® Cat. no. sc-66643), rabbit anti-Sp17 (SPA17; Proteintech™ cat. no. 13367-1-AP), mouse anti-Sp17 (EP6496; Abcam® cat. No. 172626), or mouse anti-hSp17 (A-12; SantaCruz Cat. No. sc-365325).

[000127] *Analysis of in vitro antitumor activity that is provided by different anti-sp17 agents*

[000128] M4-ID8 cells or the human ovarian cancer cell line SKOV3 (ATCC cat. No. HTB-77™) were seeded at 5000 cells/well. As a control, either naïve mouse serum or isotype–matching antibodies were used as control samples at the same concentration of anti-Sp17 agents .Each condition was tested in triplicate. Cell viability at 24 hours was determined by the calcein release assay. In brief, 24h after exposure to the anti-Sp17 agent or control agent, cells were washed and Calcein AM (2 µg/ml; ThermoFisher) was added to each well (100 µl/well in PBS). After a 30 minute incubation, the release of fluorescent calcein into the culture was measured on a plate reader set at 485 nm for excitation and 530 nm for emission.

[000129] *Analysis of sp17 epitopes that are targeted by different anti-sp17 antibodies*

[000130] Specific peptide recognition by various Sp17 antibodies from either a commercial source or provided in-house by competition ELISA. In-house antiserum (1:200 final dilution) from mice immunized with mrSp17 either adjuvanted with CpG or PSNPs, were mixed with 10 µg of each hSp17 overlapping peptides (FIG. 1), and incubated for 1 hour at room temperature. After performing the competitive binding assay, any reduction of antibody activity was measured by the ELISA method.

[000131] **Results**

[000132] The biological activities and responses described in Example 1 demonstrate that $hSp17_{111-142}$ contains sequences that are unexpectedly immunogenic and highly protective in animal models of human cancers, both in the presence or absence of an adjuvant, such as CpG. Accordingly, these sequences can be advantageously used for

preparing peptides and related pharmaceutical formulations for medical uses and methods, in particular those involving the administration of therapeutic vaccines and/or protective against cancer.

[000133] For example, in order to assess whether such $hSp17_{111-142}$ derived sequences are therapeutic, the CpG-adjuvanted, $hSp17_{111-142}$ formulation can be compared directly with CpG-adjuvanted, rmSp17 formulation in the murine ID8 model for ovarian cancer (OC). Tumor cells ($4x10^6$ ID8 tumor cells) are injected 21 days before the first therapeutic vaccination, followed by another 3 repeated treatments, 3 weeks apart. Control (naïve) mice are mock treated with PBS. Both rmSp17 full protein and $hSp17_{111-142}$ peptide treatments successfully and significantly delay tumor progression (e.g. by ~20 days; FIG. 7A). This study demonstrated the induction of anti-ID8 immunity and prolonged survival after vaccination fragment can be repeated with $hSp17_{111-142}$ fragments (e.g. those defined as $hSp17_{epoc}$), which can be synthesized and tested for the fine-mapping of B cell and Th1 cell epitope regions within the $hSp17_{111-142}$ peptide. Animals receiving either protein or peptide treatment also revealed significantly increased antibody reactivity to the ID8 lysate in serum samples, thereby demonstrating the elicitation of tumor relevant immunity (FIG. 7B).

[000134] The therapeutic efficacy for the human $hSp17_{111-142}$ peptide in a mouse OC model can be explained by the fact that the identified 9-mer strongly immunogenic B cell epitope in the human AKKMKTNSL (amino acids 134-142) and murine VKKMKSDKN (amino acids 132-140) Sp17 shares seven residues that are either identical (4 residues, shown underlined) or exhibit only a conservative change. One can test human serum samples derived from OC patients to determine if this region may also be naturally a strong immunogenic one n the course of the disease. The identified T cell epitopes KEKEEVAAVKIQAA (amino acids 111-124) and IQAAFRGIAREEAKKMK (amino acids 121-138) also contain conservative changes and stretches of identity with their murine equivalents (REQEEAAALKIQSL and IQSLFRGHVAREEVKKMK), which are useful in human studies as well as the sequence of the IQ motif EVAAVKIQAAFRGIAREE (calmodulin-binding recognition sequence) that is present in hSp17 (amino acids 115-133; Wen et al., 1999).

[000135] These results further support the use of the disclosed $hSp17_{111-142}$ peptide not only as an effective vaccine, but also as an epitope platform for defining antigen-specific antibodies useful for cancer immunotherapy that are present in the sera of mice immunized with this peptide, as described in Example 1 herein (see also FIG. 3 and FIG. 5).

[000136] Initially, in order to determine whether eliminating only the Sp17-positive ID8 ovarian cancer OC cells (in cell culture lines such as ID8, which also contain Sp17- cells) would eliminate their capacity to cause tumors, the ID8 cells were further cloned by limiting dilution method, and tested for clones exhibiting Sp17 expression by flow cytometry. The Sp17 positive (Sp17+) and negative (Sp17-) subsets were grown as clones, and characterized *in vitro* before implantation in animals and further selection. The examined cell populations showed similar growth rate in culture (FIG. 7C). The more tumorigenic clonal population (the M4-ID8 cells) was analyzed on the basis of Sp17 co-expression with two important markers for ovarian cancer (OC): PD-L1 and STAT3. OC patients showing higher expression of PDL1 on OC cells have poorer prognosis than those with lower expression (Hamanishi J et al., 2007) and the expression of PDL1 on tumor cells has been associated with tumor escape (Iwai Y et al., 2002). The transcription factor STAT3 has a dual role in cancer, but confers resistance to chemotherapy and other cancer drugs to OC cells (Duan Z et al., 2006). In M4-ID8 cells, Sp17 is co-expressed with high levels of PDL1 and STAT3 (FIG. 7D). Therefore, targeting Sp17-positive cells would mean simultaneously targeting cancer cells associated with resistance to chemotherapy and resistance to destruction by T cells or NK cells.

[000137] These findings therefore provide a sound basis underscoring the importance of Sp17 expression for tumor progression *in vivo* in immunocompetent animals. Indeed, Sp17-positive and Sp17-negative ID8 cells have clear and dramatic differences in tumorigenic activity *in vivo,* with the latter ones completely failing to induce OC tumors *in vivo* even when a higher number of cells were initially inoculated compared to Sp17-positive ID8 cells (FIG. 7E; reflecting data collected up to 153 days). Use of an ovarian cancer model based on a highly tumorigenic, Sp17-positive ID8 variant (M4-ID8) that is selected to effectively grow *in vivo* can also facilitate the characterization of Sp17-based therapies (such as $hSP17_{111-142}$-based vaccines or antibodies), which reveals cancer formation within a month, whilst recapitulating the hallmark features of the most lethal forms of human OC, including ascites formation as early as Day 18. By Day 28, cancer nodules in the diaphragm, liver lobe, spleen, stomach, and floating nodules in the abdominal area are evident (FIG. 7F).

[000138] Sp17 appears therefore an essential feature for cancerous tumor growth *in vivo*, especially for ovarian cancer. This property can be exploited in various ways, for instance for combining Sp17-targeting agents with other treatments to eliminate the most forms of aggressive cancer cells and/or for targeting potential residual Sp17-

negative cancer cells after targeting Sp17-positive cancer cells. Indeed, the presence of antibodies against $hSp17_{111-142}$ in OC-bearing animals that responded to the therapeutic vaccination with $hSp17_{111-142}$ (Example 1), revealed that such antibodies might directly promote the specific killing of tumor cells.

[000139] Potential $Sp17_{epoc}$-based agents have been characterized by using sera and commercial anti-human Sp17 antibodies. To test for direct OC killing potential, we compared sera from healthy unimmunized mice with mice immunized with $hSp17_{111-142}$ for their capacity to directly kill OC cells *in vitro*. Sera from mice administered with either CpG- or PSNPs-adjuvanted $hSp17_{111-142}$ and containing anti- $hSp17_{111-142}$ antibodies, but not control sera, were shown to directly kill both ID8 mouse ovarian cancer cells and human ovarian cancer SKOV3 cells (FIG. 8A). When $hSp17_{111-142}$-based sera are compared with sera generated by immunizing mice with full human Sp17-sequence antibodies within such full hSp17-generated control sera showed high specificity for Sp17 epitopes other than $hSp17_{111-142}$ (FIG. 8B). If the same analysis of antitumor activity and Sp17 epitope binding is performed with commercially available antibodies (FIG. 8C and FIG. 8D), anti-hSp17 antibody specificities other than for $hSp17_{111-142}$ clearly fail to confer antitumor properties to such antibodies. By using full Sp17 or Sp17 fragments in the N-terminal or central region of the proteins, the resulting anti-hSp17 for Sp17 antibodies (and the hybridoma cells that can be isolated) are useful for hSp17 immunodetection, but not as anti-cancer agents. Indeed, the Sp17 epitope mapping for commercial antibodies indicates a strong preference for epitopes located in Sp17-derived peptides other than $hSp17_{111-142}$ (such as $hSp17_{1-32}$, $hSp17_{23-54}$, $hSp17_{45-76}$, and/or $hSp17_{67-98}$ peptides).

[000140] The present data permit the identification of improved anti-Sp17 agents for treating cancers such as ovarian cancer, in particular by using monoclonal antibodies that selectively target cells exposing cancer-specific human Sp17 epitopes (anti-$Sp17_{epoc}$). Anti-$Sp17_{epoc}$ can be suitably used as anti-cancer agents alone or in combination with other anti-cancer agents that target other cancer-relevant antigens (such as other cell surface antigens, immune checkpoint inhibitors, etc.) radio- or chemotherapy, or other anti-cancer drugs, such as those listed in WHO Model List of Essential Medicines (19^{th} edition, April 2015). Variable regions or selected CDR sequences from anti-$Sp17_{epoc}$ can be integrated in protein scaffolds or modules that are based on natural immunoglobulins (such as full IgG scFV, Nanobodies, Fab, etc.) or non-immunoglobulin scaffolds for protein binding (such as affibodies, DARPins, Anticalin, Monobody, etc.).

[000141] Anti-hSp17$_{epoc}$ may be used for treating cancers including ovarian cancer (OC), which is the leading cause of death from gynecological malignancy, which requires new therapeutic approaches. Different from known targeted therapies involving cancer cell specific monoclonal antibodies that can increase patient survival for breast, lung, and other cancer indications, only a limited proportion of OC cells presents specifically express cell surface targets such as those commonly found with other cancers, such Her-2/neu or MUC1. Additionally, OC cell variability within an individual patient, typically leads to growth of drug resistant cells following chemotherapy and cellular evasion of immune mechanisms of tumor elimination (Chester, C., et al., 2015; Lloyd K et al., 2015).

[000142] Anti-hSp17$_{epoc}$ may also broadly target OC patients, and specifically target elements of OC cells crucial to their *in vivo* survival and for their ability to escape other known therapeutic approaches, by eliminating the cells most likely to cause untreatable recurrences. Moreover, anti-Sp17$_{epoc}$ may target cancer cells in which hSp17$_{epoc}$ is present with proteins associated with drug resistance like STAT3 (Lee H-J et al., 2014) or immune evasion, like PDL1 (Adachi K and Tamada K, 2015; Beatty G et al., 2015). In parallel to vaccination strategies, targeting hSp17epoc in a highly aggressive immunocompetent OC mouse model that closely mimics the most lethal type of spontaneous human epithelial OC, the present invention demonstrates how sera, raised using hSp17$_{epoc}$, and thus enriched in anti-hSp17$_{epoc}$ antibodies, is also very effective in directly targeting and destroying murine and human OC cells. This particular result is not obtainable using presently available commercial monoclonal antibodies that detect hSp17 via other regions, which have been shown to be otherwise ineffective. The inventive aspects disclosed herein can be advantageously exploited for developing a variety of new therapeutic strategies against tumors, including tumors associated with ovarian cancer, whereby such strategies beneficially overcome the often toxic and not fully effective therapeutic regimens used currently.

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CLAIMS

1. A fragment of human sperm surface protein Sp17 (hSp17) corresponding to amino acids 111-142 of the human Sp17 protein ($hSp17_{111-142}$; SEQ ID NO: 1).

2. A peptide derived from the peptide of claim 1, comprising 1, 2, 3, or more amino acid substitutions.

3. A peptide derived from the peptide of claim 1 or 2, wherein said peptide comprises from 10 to 31 amino acids.

4. Use of a peptide of any of claims 1 to 3 in the preparation of an anti-cancer vaccine composition.

5. A pharmaceutical composition comprising a peptide according to any of the claims 1 to 3.

6. Methods for treating or preventing cancer comprising the administration of a peptide according to any of claims 1 to 3.

7. An antigen-binding fragment binding a peptide of any of the claims 1 to 3.

8. The antigen binding fragment of claim 7, wherein said fragment is comprised in a monoclonal antibody.

9. The antigen binding fragment of claim 7, wherein said fragment is comprised in human or mouse sera.

10. The antigen binding fragment of claim 7, wherein said fragment is comprised in an oligoclonal or polyclonal antibody preparation.

11. The antigen binding fragment of claim 7, wherein said fragment is comprised in construct together with antigen-binding sequences that are antigen-binding sequences that bind another cancer target selected from checkpoint inhibitors or other antigen characterizing cancer cellss.

12. A host cells expressing the antigen binding fragment of claim 7.

13. An hybrdoma expressing the monoclonal antibody of claim 8.

14. Use of a antigen-binding fragment of any of claims 7 to 10 in the preparation of an anti-cancer antibody composition.

15. A pharmaceutical composition comprising an antigen-binding fragment of any of claims 7 to 10.

16. Methods for treating or preventing cancer comprising the administration of an antigen-binding fragment of any of claims 7 to 10.

17. The method of claim 16 wherein the administration of an antigen-binding fragment is in combination with the administration of chemotherapeutic agents, radiotherapy, immunotherapeutic agents, and/or inhibitors of kinases.

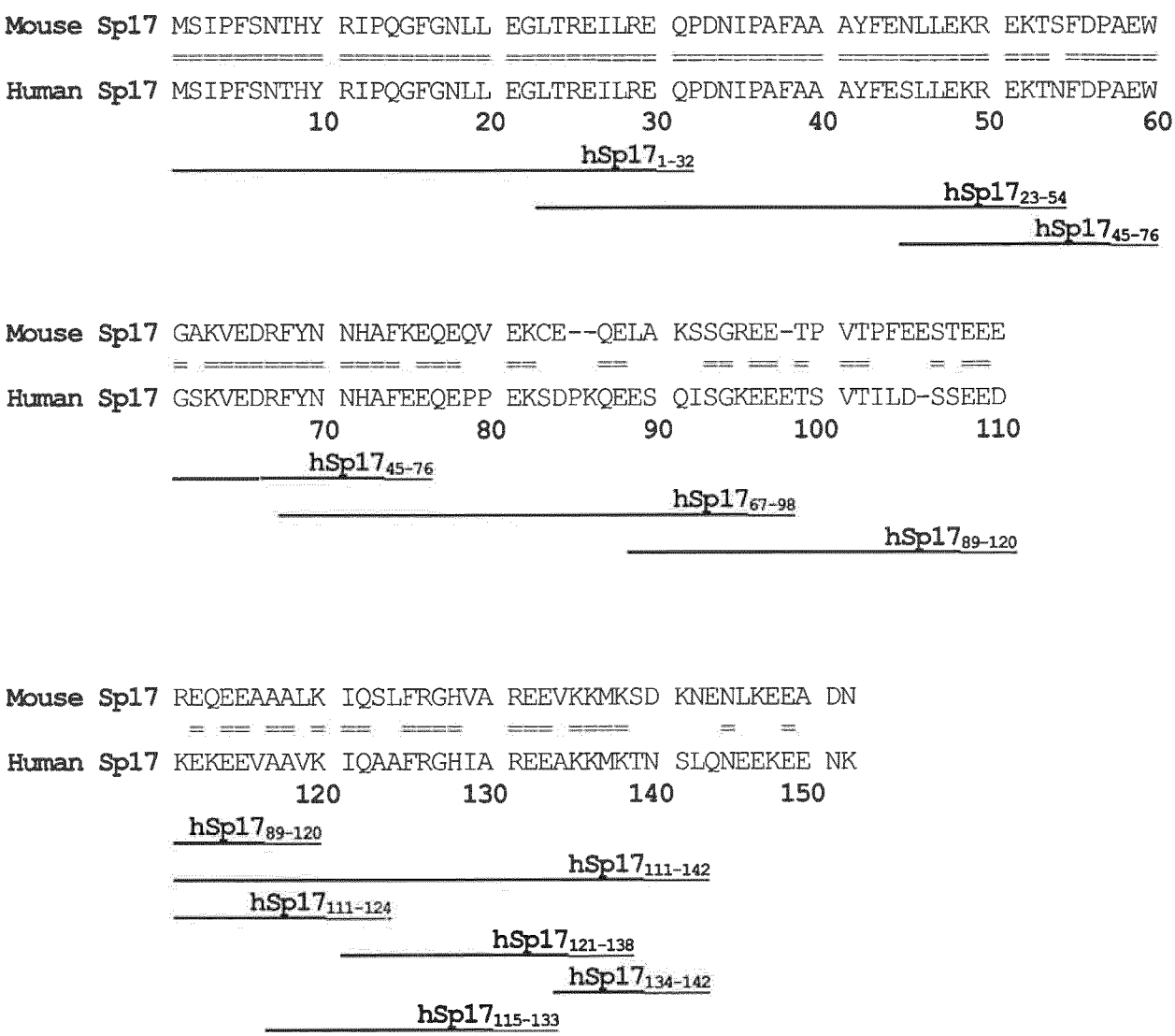

Mouse Sp17 MSIPFSNTHY RIPQGFGNLL EGLTREILRE QPDNIPAFAA AYFENLLEKR EKTSFDPAEW
Human Sp17 MSIPFSNTHY RIPQGFGNLL EGLTREILRE QPDNIPAFAA AYFESLLEKR EKTNFDPAEW
10 20 30 40 50 60
hSp17 1-32
hSp17 23-54
hSp17 45-76
Mouse Sp17 GAKVEDRFYN NHAFKEQEQV EKCE--QELA KSSGREE-TP VTPFEESTEEE
Human Sp17 GSKVEDRFYN NHAFEEQEPP EKSDPKQEES QISGKEEETS VTILD-SSEED
70 80 90 100 110
hSp17 45-76
hSp17 67-98
hSp17 89-120
Mouse Sp17 REQEEAAALK IQSLFRGHVA REEVKKMKSD KNENLKEEA DN
Human Sp17 KEKEEVAAVK IQAAFRGHIA REEAKKMKTN SLQNEEKEE NK
120 130 140 150
hSp17 89-120
hSp17 111-142
hSp17 111-124
hSp17 121-138
hSp17 134-142
hSp17 115-133

FIG. 1

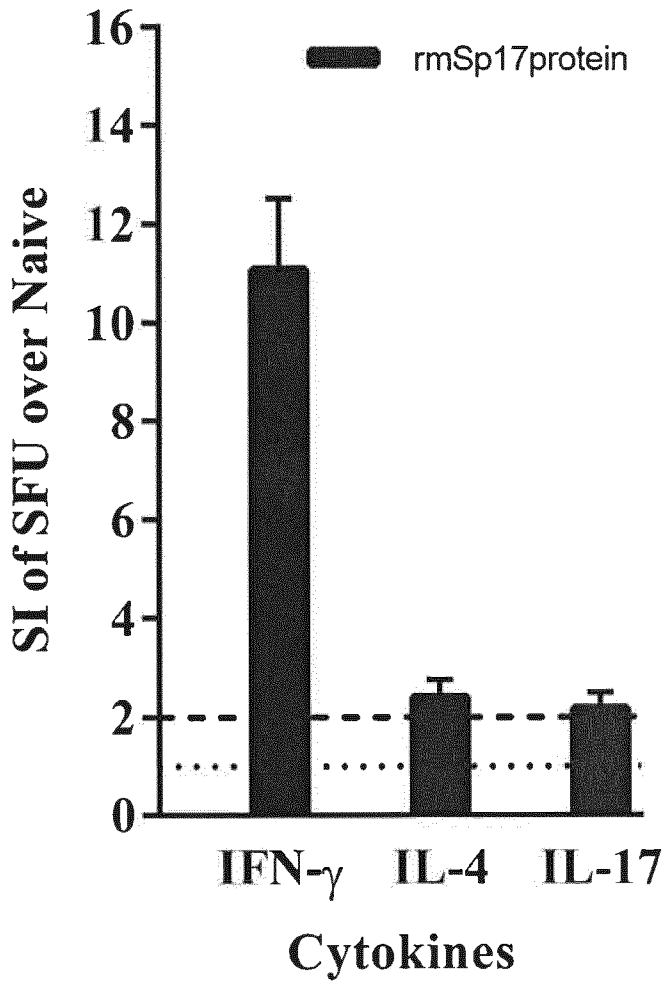

rmSp17protein
SI of SFU over Naive
16
14
12
10
8
6
4
2
0
IFN-γ
IL-4
IL-17
Cytokines

FIG. 2

FIG. 3A IFN-γ response

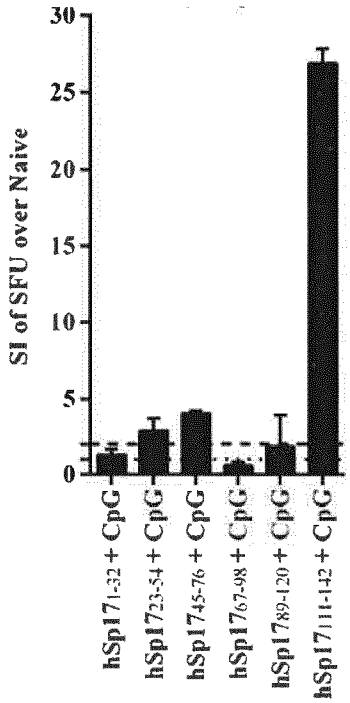


FIG. 3B Antigen specific antibody production by CpG adjuvanted hSp17 peptide formulations

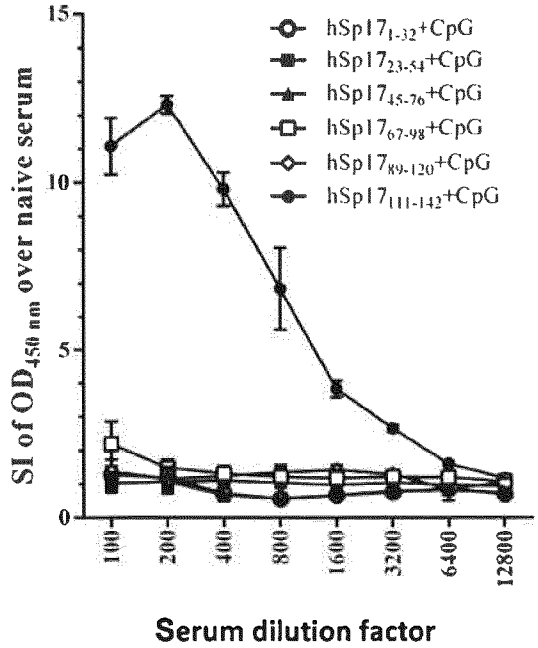


FIG. 3C

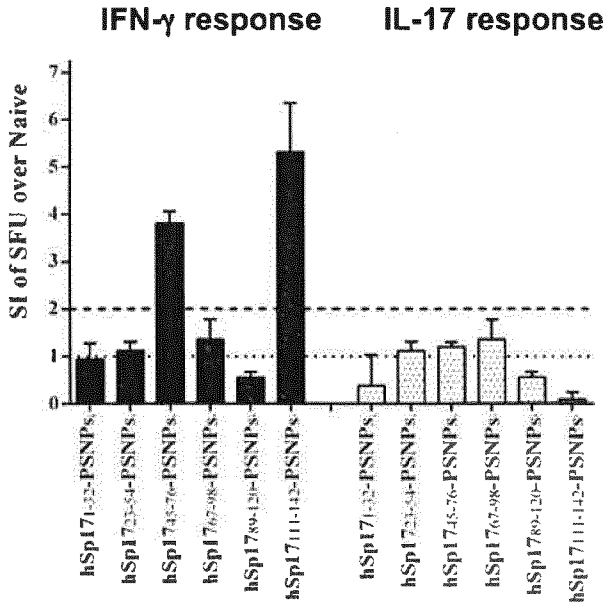


FIG. 3D Antigen specific antibody production by PSNPs adjuvanted hSp17 peptide formulations

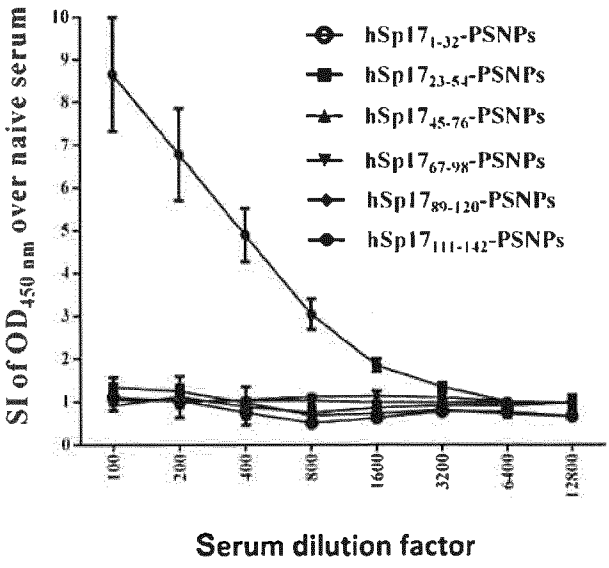


FIG. 4A

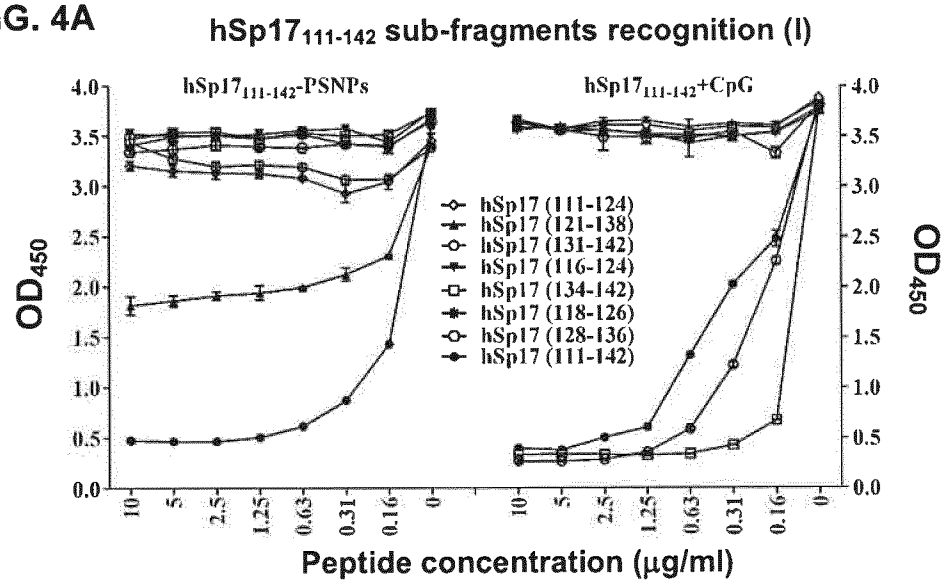


FIG. 4B

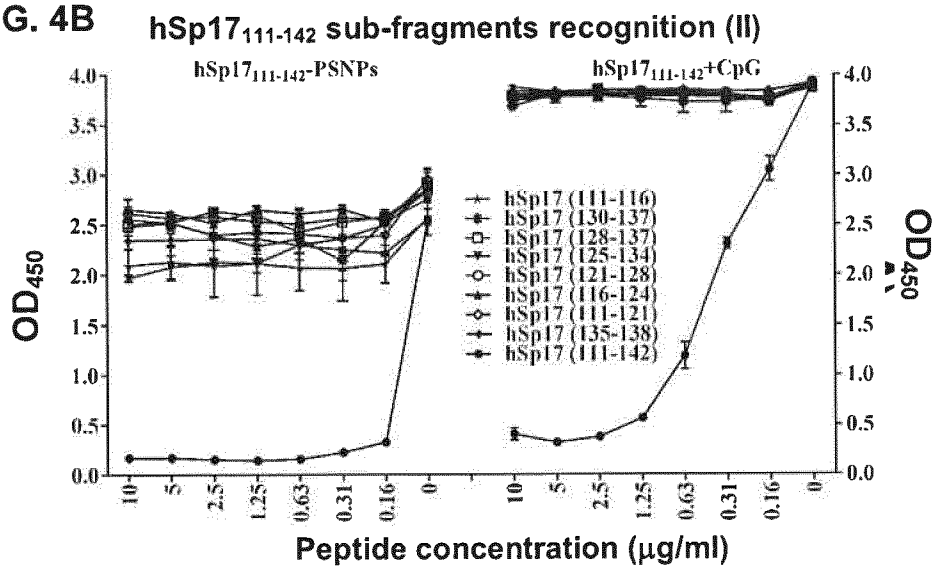


FIG. 4C Cross-species antibody recognition of mSp17 sub-fragments

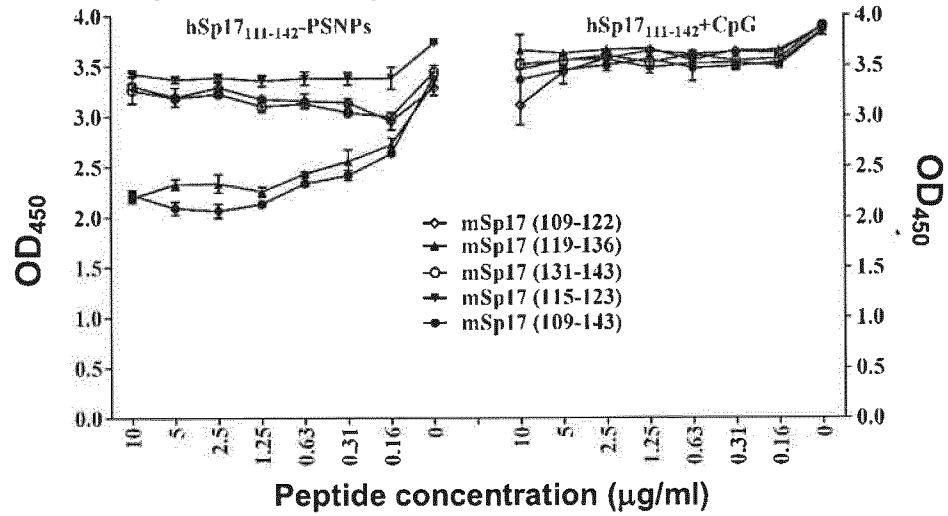


FIG. 5A IFN-γ response

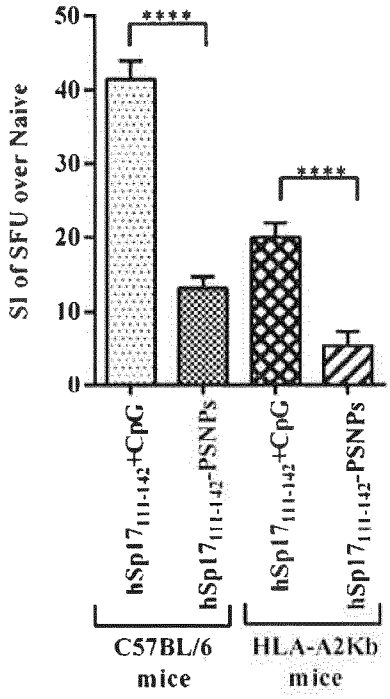


FIG. 5B Anti-hSp17$_{111-142}$ antibody

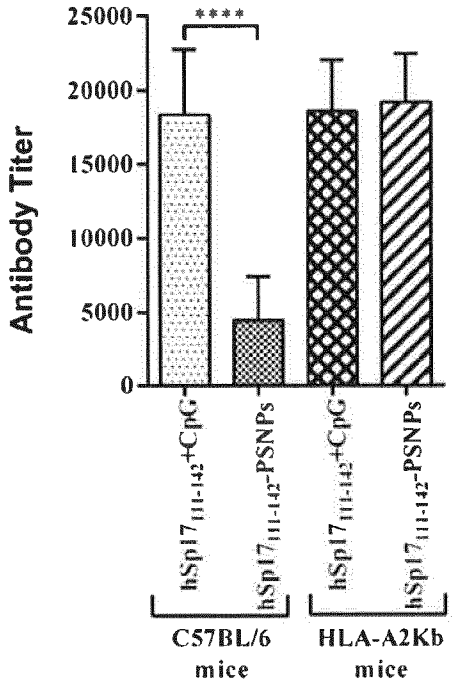


FIG. 5C IgG subtypes induced by hSp17$_{111-142}$ peptide formulations adjuvanted by CpG and PSNPs

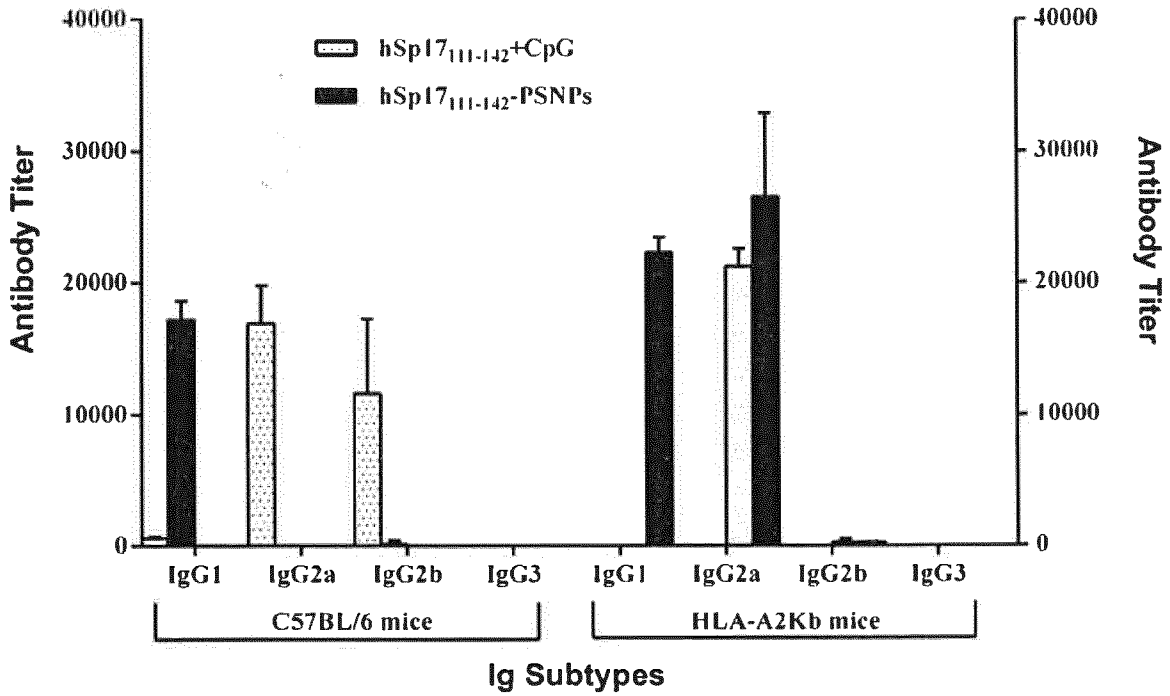


FIG. 6A

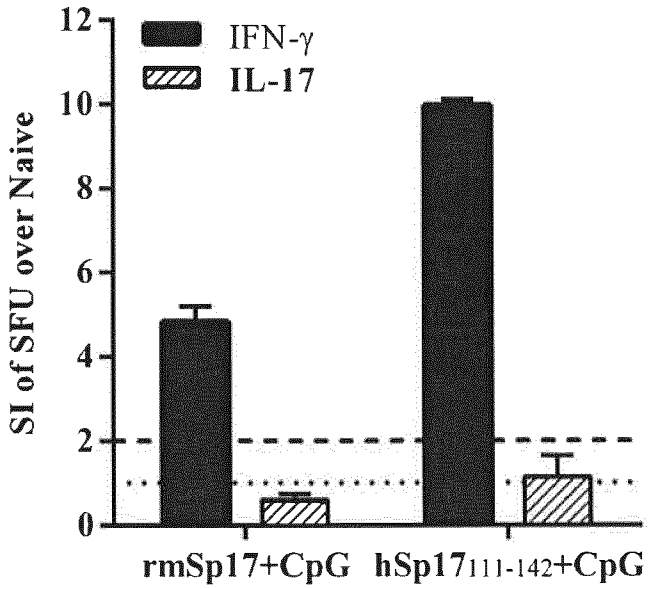

IFN-γ
IL-17
SI of SFU over Naive
12
10
8
6
4
2
0
rmSp17+CpG
hSp17111-142+CpG

FIG. 6B

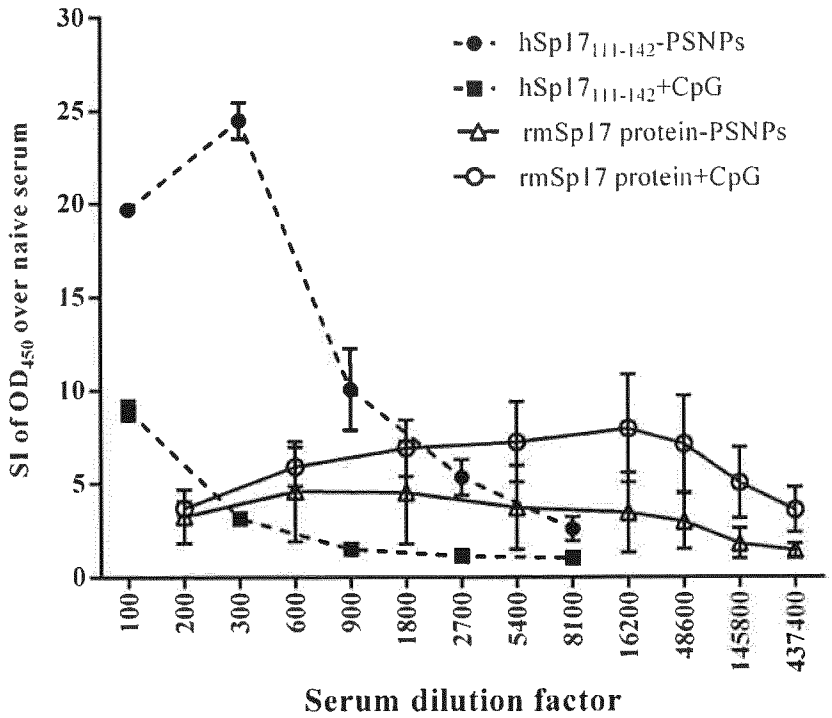

hSp17111-142-PSNPs
hSp17111-142+CpG
rmSp17 protein-PSNPs
rmSp17 protein+CpG
SI of OD450 over naive serum
30
25
20
15
10
5
0
100
200
300
600
900
1800
2700
5400
8100
16200
48600
145800
437400
Serum dilution factor

FIG. 7A

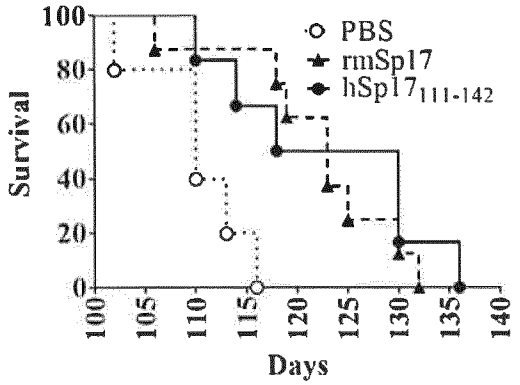


FIG. 7B

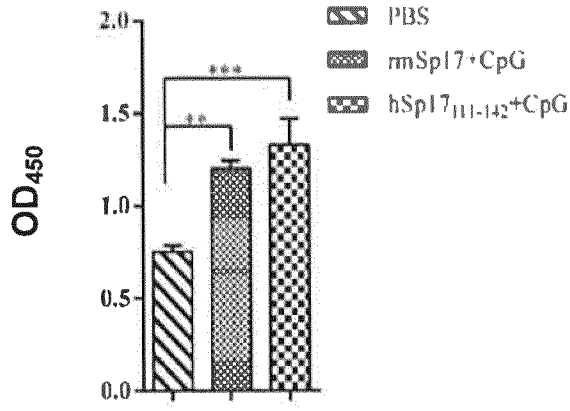


FIG. 7C

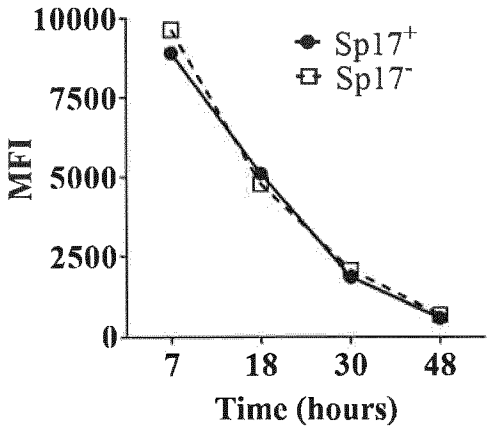


FIG. 7D

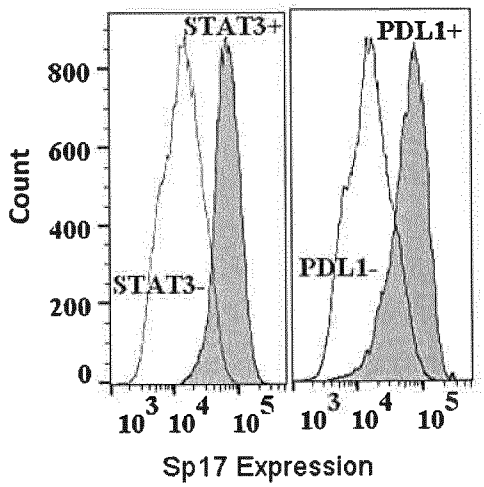


FIG. 7E

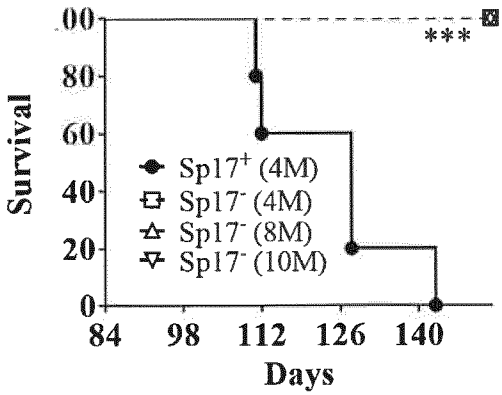


FIG. 7F

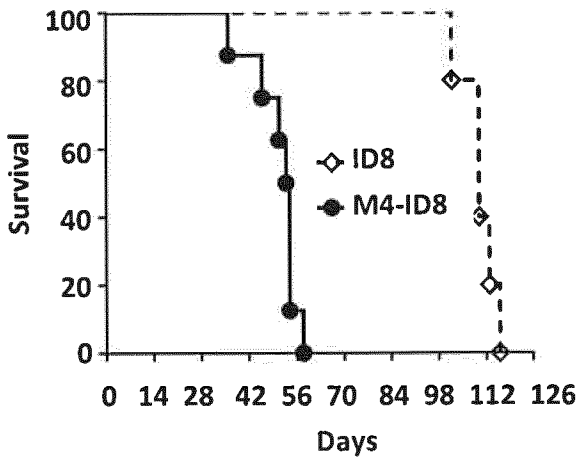


FIG. 8A

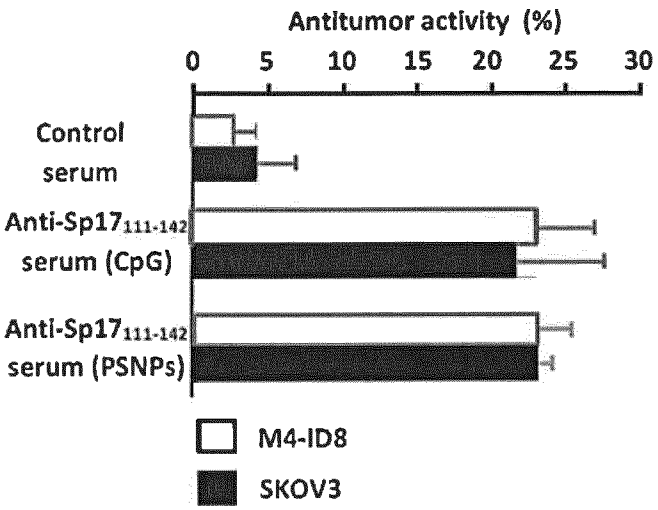


FIG. 8B

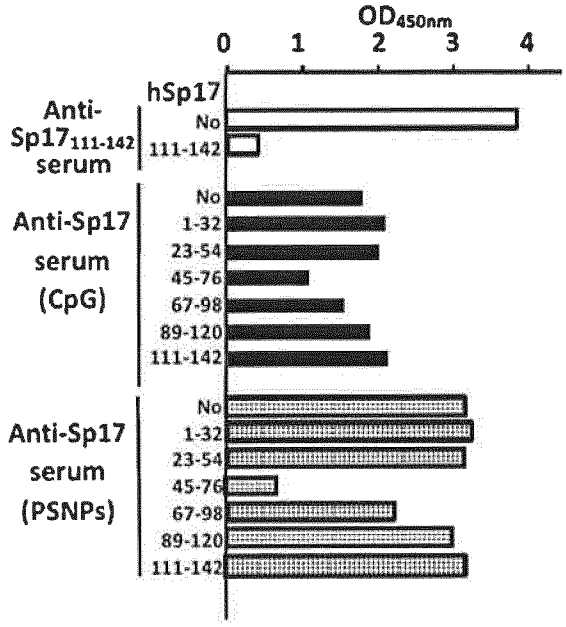


FIG. 8C

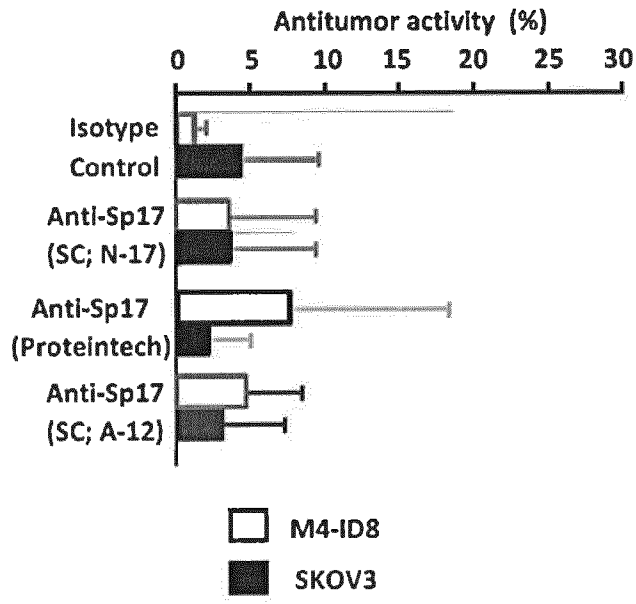
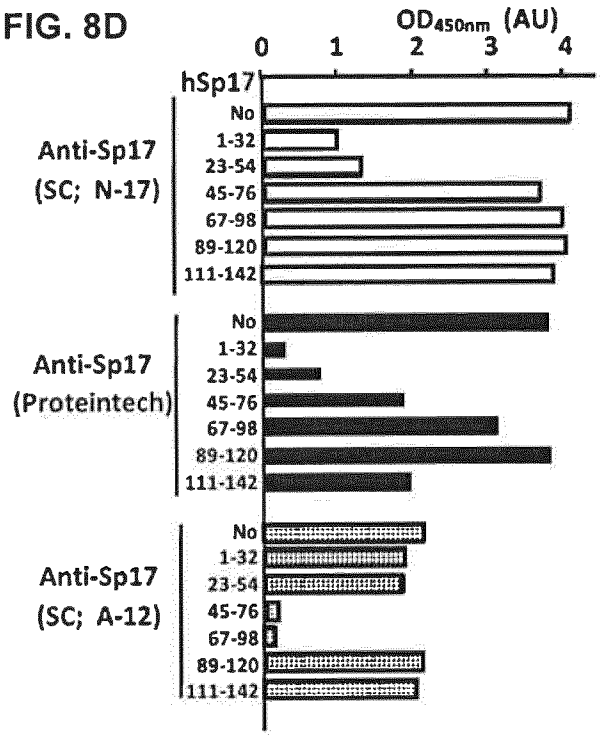


FIG. 8D



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/067468

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/705 C07K16/00
ADD.

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)
C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/15764 A1 (UNIV NORTH CAROLINA [US]; RAND MICHAEL G O [US]; WIDGREN ESTHER E [US]) 15 June 1995 (1995-06-15) Sequence ID AAR79761 -----	1-3,5, 7-13
Y	DADABAYEV ALISHER R ET AL: "Cancer immunotherapy targeting Sp17: When should the laboratory findings be translated to the clinics?", AMERICAN JOURNAL OF HEMATOLOGY, vol. 80, no. 1, September 2005 (2005-09), pages 6-11, XP002763428, ISSN: 0361-8609 page 7, right-hand column - page 9, right-hand column, paragraph 1 ----- -/--	1-17

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
2 December 2016	16/12/2016

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

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PCT/EP2016/067468

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