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(54) Title: TARGET PEPTIDES FOR CANCER THERAPY AND DIAGNOSTICS

(57) Abstract: A set of target peptides are presented by HLA class I molecules on the surface of hepatocellular carcinoma (HCC) ceils and/or esophageal cancer cells. They are envisioned to among other things (a) stimulate an immune response to the proliferative disease, *e.g.*, HCC and/or esophageal cancer, (b) function as immunotherapeutics in adoptive T-cell therapy or as a vaccine, (c) facilitate antibody recognition of tumor boundaries in surgical pathology samples, (d) act as biomarkers for early detection and/or diagnosis of the disease, and (e) act as targets in the generation antibody-like molecules which recognize the target-peptide/MHC complex.

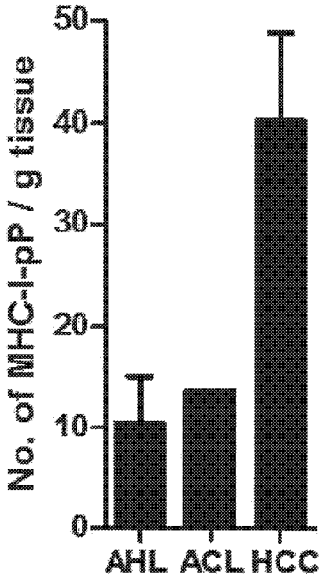

FIG. 1A

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[Continued on next page]

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DESCRIPTION

TARGET PEPTIDES FOR CANCER THERAPY AND DIAGNOSTICS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application Serial No. 62/332,139, filed May 5, 2016, the disclosure of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

The Sequence Listing associated with the instant disclosure has been electronically submitted to the United States Patent and Trademark Office as a 137 kilobyte ASCII text file created on May 3, 2017 and entitled "3062_13_PCT_ST25.txt". The Sequence Listing submitted via EFS-Web is hereby incorporated by reference in its entirety.

GRANT STATEMENT

This invention was made with government support under Grant No. AI033993 awarded by National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

The presently disclosed subject matter relates to diagnostics and therapeutics. In particular, it relates to immunotherapies and diagnostics in the context of proliferative diseases such as cancer.

BACKGROUND

The mammalian immune system has evolved a variety of mechanisms to protect the host from cancerous cells. An important component of this response is mediated by cells referred to as T cells. Cytotoxic T lymphocytes (CTL) are specialized T cells that primarily function by recognizing and killing cancerous cells or infected cells, but they can also function by secreting soluble molecules referred to as cytokines that can mediate a variety of effects on the immune system. T helper cells primarily function by recognizing antigen on specialized antigen presenting cells, and in turn secreting cytokines that activate B cells, T cells, and macrophages. A variety of evidence suggests that immunotherapy designed to stimulate a tumor-specific CTL response would be effective in controlling cancer. For example, it has been shown that human CTL recognize sarcomas (Slovin *et al.*, 1986), renal cell carcinomas (Schendel *et al.*, 1993), colorectal carcinomas (Jacob *et al.*, 1997), ovarian carcinomas (Peoples *et al.*, 1993), pancreatic carcinomas (Peiper *et al.*, 1997), squamous tumors of the head and neck (Yasumura *et al.*, 1993), and squamous carcinomas of the lung (Slingluff *et al.*, 1994; Yoshino *et al.*, 1994). The largest

number of reports of human tumor-reactive CTLs, however, has concerned melanomas (Boon *et al.*, 1994). The ability of tumor-specific CTL to mediate tumor regression, in both human (Parmiani *et al.*, 2002; Weber, 2002) and animal models, suggests that methods directed at increasing CTL activity would likely have a beneficial effect with respect to tumor treatment.

Liver Cancer (hepatocellular carcinoma, HCC) is the sixth most common cancer in the world. Incidence and mortality are growing in Europe and most parts of the world. Chronic liver diseases predispose for the development of HCC (liver cirrhosis of any etiology, alcoholic liver disease, chronic viral infection, autoimmunehepatitis, etc.). Unfortunately, diagnosis is often made in late stages of the disease and to this day only very limited treatment options are available for HCC, especially in advanced stage disease (Llovet *et al.*, 2012). Since HCC has been shown to be immunogenic (Wada *et al.*, 1998; Takayama *et al.*, 2000; Parmiani & Anichini, 2006), immunotherapy is considered to be a promising new treatment modality. The identification of novel and specific tumor antigens provides the basis for the development of an efficient anti-cancer immunotherapy. Only a few HCC-specific tumor antigens have been characterized so far (Breous & Thimme, 2011; Buonaguro *et al.*, 2013), although it has been shown that up to 10.000 different peptides can be presented with MHC-I-molecules on the surface of tumor cells (Zarling *et al.*, 2000).

Esophageal cancer is also a leading cause of death from cancer worldwide. The two principal types of esophageal cancer are squamous cell carcinoma and adenocarcinoma. Both are relatively uncommon in the U.S., comprising approximately 1% of all cancers. However, the incidence of adenocarcinoma is rising at a rapid rate. The 5-year survival rates for localized and all stages combined are 34% and 17%, respectively. Moreover, there is no currently reliable method for early detection or for the prediction of treatment outcome.

Barrett's esophagus (BE), high-grade dysplasia (HGD), and invasive cancer are thought to comprise a multi-step process in the development of esophageal adenocarcinoma (EAC or OEAC). HGD has been considered as the immediate precursor of invasive adenocarcinoma, and most patients with HGD develop cancer. No intervention currently exists that prevents the progression of BE or HGD to esophageal cancer. The traditional methods for diagnosing esophageal cancer include endoscopy and barium swallow, but the poor specificity and sensitivity of these methods results in their detection

only at an advanced stage. Recently however, prognostic and predictive markers have been identified that aid in the diagnosis of esophageal cancer.

Alteration in the phosphorylation status of cellular signaling proteins is a hallmark of malignant transformation. This altered phosphorylation status leads to up- or downregulation of signaling pathways, which are indispensable for tumor growth. Deregulated phosphorylation can create neoantigens that bind to major histocompatibility complex (MHC) molecules and the phosphorylation affects the antigenic identity of the presented epitopes (Mohammed *et al.*, 2008). It has been shown that phosphoproteins are processed and presented on tumor cells and that they are recognized by the immune system in a phosphorylation-dependent manner (Zarling *et al.*, 2006). Further studies revealed that MHC class-I molecules seem to have a higher affinity towards the phosphorylated peptide in comparison to the unphosphorylated counterpart and that the phosphate group is exposed outwards in direction to the T cell receptor (TCR) in order to improve contact with the TCR (Mohammed *et al.*, 2008, *see particularly* Figure 1 therein). The phosphoproteome therefore seems to be an attractive target for cancer immunotherapy (Zarling *et al.*, 2000; Zarling *et al.*, 2006; Mohammed *et al.*, 2008; Cobbold *et al.*, 2013).

In order for CTL to kill or secrete cytokines in response to a cancer cell, the CTL must first recognize the cancer cell (Townsend & Bodmer, 1989). This process involves the interaction of the T cell receptor, located on the surface of the CTL, with what is generically referred to as an MHC-peptide complex which is located on the surface of the cancerous cell. MHC (major histocompatibility-complex)-encoded molecules have been subdivided into two types, and are referred to as class I and class II MHC-encoded molecules. In the human immune system, MHC molecules are referred to as human leukocyte antigens (HLA). Within the MHC complex, located on chromosome six, are three different loci that encode for class I MHC molecules. MHC molecules encoded at these loci are referred to as HLA-A, HLA-B, and HLA-C. The genes that can be encoded at each of these loci are extremely polymorphic, and thus, different individuals within the population express different class I MHC molecules on the surface of their cells. HLA-A1, HLA-A2, HLA-A3, HLA-B7, HLA-B14, HLA-B27, and HLA-B44 are examples of different class I MHC molecules that can be expressed from these loci.

The peptides which associate with the MHC molecules can either be derived from proteins made within the cell, in which case they typically associate with class I MHC molecules (Rock & Goldberg, 1999); or they can be derived from proteins which are acquired from outside of the cell, in which case they typically associate with class II MHC

molecules (Watts, 1997). The peptides that evoke a cancer-specific CTL response most typically associate with class I MHC molecules. The peptides themselves are typically nine amino acids in length, but can vary from a minimum length of eight amino acids to a maximum of fourteen amino acids in length. Tumor antigens can also bind to class II MHC molecules on antigen presenting cells and provoke a T helper cell response. The peptides that bind to class II MHC molecules are generally twelve to nineteen amino acids in length, but can be as short as ten amino acids and as long as thirty amino acids.

The process by which intact proteins are degraded into peptides is referred to as antigen processing. Two major pathways of antigen processing occur within cells (Rock & Goldberg, 1999). One pathway, which is largely restricted to professional antigen presenting cells such as dendritic cells, macrophages, and B cells, degrades proteins that are typically phagocytosed or endocytosed into the cell. Peptides derived from this pathway can be presented on either class I or to class II MHC molecules. A second pathway of antigen processing is present in essentially all cells of the body. This second pathway primarily degrades proteins that are made within the cells, and the peptides derived from this pathway primarily bind to class I MHC molecules. Antigen processing by this latter pathway involves polypeptide synthesis and proteolysis in the cytoplasm, followed by transport of peptides to the plasma membrane for presentation. These peptides, initially being transported into the endoplasmic reticulum of the cell, become associated with newly synthesized class I MHC molecules and the resulting complexes are then transported to the cell surface. Peptides derived from membrane and secreted proteins have also been identified. In some cases these peptides correspond to the signal sequence of the proteins which is cleaved from the protein by the signal peptidase. In other cases, it is thought that some fraction of the membrane and secreted proteins are transported from the endoplasmic reticulum into the cytoplasm where processing subsequently occurs. Once bound to the class I MHC molecule, the peptides are recognized by antigen-specific receptors on CTL. Several methods have been developed to identify the peptides recognized by CTL, each method of which relies on the ability of a CTL to recognize and kill only those cells expressing the appropriate class I MHC molecule with the peptide bound to it. Mere expression of the class I MHC molecule is insufficient to trigger the CTL to kill the target cell if the antigenic peptide is not bound to the class I MHC molecule. Such peptides can be derived from a non-self source, such as a pathogen (for example, following the infection of a cell by a bacterium or a virus) or from a self-derived protein within a cell, such as a cancerous cell. The tumor antigens from which the peptides

are derived can broadly be categorized as differentiation antigens, cancer/testis antigens, mutated gene products, widely expressed proteins, viral antigens and most recently, phosphopeptides derived from dysregulated signal transduction pathways. (Zarling *et al.*, 2006).

Immunization with HCC-derived, class I or class II MHC-encoded molecule associated peptides, or with a precursor polypeptide or protein that contains the peptide, or with a gene that encodes a polypeptide or protein containing the peptide, are forms of immunotherapy that can be employed in the treatment of HCC. Identification of the immunogens is a necessary first step in the formulation of the appropriate immunotherapeutic agent or agents. Although a large number of tumor-associated peptide antigens recognized by tumor reactive CTL have been identified, there are few examples of antigens that are derived from proteins that are selectively expressed on a broad array of tumors, as well as associated with cellular proliferation and/or transformation.

Attractive candidates for this type of antigen are peptides derived from proteins that are differentially phosphorylated on serine (Ser), threonine (Thr), and tyrosine (Tyr; Zarling *et al.*, 2000). Due to the increased and dysregulated phosphorylation of cellular proteins in transformed cells as compared to normal cells, tumors are likely to present a unique subset of phosphorylated peptides on the cell surface that are available for recognition by cytotoxic T-lymphocytes (CTL). Presently, there is no way to predict which protein phosphorylation sites in a cell will be unique to tumors, survive the antigen processing pathway, and be presented to the immune system in the context of phosphopeptides bound to class I MHC molecules.

SUMMARY

This Summary lists several embodiments of the presently disclosed subject matter, and in many cases lists variations and permutations of these embodiments. This Summary is merely exemplary of the numerous and varied embodiments. Mention of one or more representative features of a given embodiment is likewise exemplary. Such an embodiment can typically exist with or without the feature(s) mentioned; likewise, those features can be applied to other embodiments of the presently disclosed subject matter, whether listed in this Summary or not. To avoid excessive repetition, this Summary does not list or suggest all possible combinations of such features.

The presently disclosed subject matter provides in some embodiments compositions comprising at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more synthetic target peptides. In some embodiments, each synthetic target peptide is about or at least 6,

7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids long, optionally between 8 and 50 amino acids long; and comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-448 and 502-529, and further wherein said composition optionally has the ability to stimulate a T cell-mediated immune response to at least one of the synthetic target peptides and/or is capable of eliciting a memory T cell response to at least one of the synthetic target peptides. In some embodiments, the synthetic target peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, 502, and 509-529. In some embodiments, at least one of the synthetic target peptides comprises a substitution of a serine residue with a homo-serine residue. In some embodiments, at least one of the synthetic target peptides is a phosphopeptide that comprises a non-hydrolyzable phosphate group. In some embodiments, the composition is immunologically suitable for use in a hepatocellular carcinoma (HCC) patient and/or an esophageal cancer patient. In some embodiments, the composition comprises at least 2, 3, 4, or 5 different target peptides, at least 10 different target peptides, or at least 15 different target peptides.

In some embodiments, at least one of the synthetic target peptides is capable of binding to an MHC class I molecule selected from the group consisting of an HLA-A*0201 molecule, an HLA A*0101 molecule, an HLA A*0301 molecule, an HLA B*4402 molecule, an HLA B*0702 molecule, and an HLA B*2705 molecule.

In some embodiments the composition is capable of increasing the 5-year survival rate of HCC patients and/or esophageal cancer patients treated with the composition by at least 20 percent relative to average 5-year survival rates that could have been expected without treatment with the composition. In some embodiments, the composition is capable of increasing the survival rate of HCC and/or esophageal cancer patients treated with the composition by at least 20 percent relative to a survival rate that could have been expected without treatment with the composition. In some embodiments, the composition is capable of increasing the treatment response rate of HCC and/or esophageal cancer patients treated with the composition by at least 20 percent relative to a treatment rate that could have been expected without treatment with the composition. In some embodiments, the

composition is capable of increasing the overall median survival of patients of HCC and/or esophageal cancer patients treated with the composition by at least two months relative to an overall median survival that could have been expected without treatment with the composition.

In some embodiments, the presently disclosed compositions further comprise at least one peptide derived from MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGE), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, β-HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein/cyclophilin C-associated protein), TAAL6, TAG72, TLP, and TPS.

In some embodiments, the presently disclosed compositions further comprise an adjuvant selected from the group consisting of montanide ISA-51, QS-21, a tetanus helper peptide, GM-CSF, cyclophosamide, bacillus Calmette-Guerin (BCG), corynbacterium parvum, levamisole, azimezone, isoprinisone, dinitrochlorobenezene (DNCB), keyhole limpet hemocyanin (KLH), complete Freunds adjuvant, in complete Freunds adjuvant, a mineral gel, aluminum hydroxide (Alum), lysolecithin, a pluronic polyol, a polyanion, an adjuvant peptide, an oil emulsion, dinitrophenol, and diphtheria toxin (DT), or any combination thereof.

In some embodiments, the presently disclosed compositions comprise a peptide capable of binding to an MHC class I molecule selected from the group consisting of an HLA-A*0201 molecule, an HLA A*0101 molecule, an HLA A*0301 molecule, an HLA B*4402 molecule, an HLA B*0702 molecule, and an HLA B*2705 molecule.

In some embodiments of the presently disclosed compositions, at least one of the synthetic target peptides is phosphorylated on a serine residue, a threonine residue, a tyrosine residue, or any combination thereof.

In some embodiments, the presently disclosed compositions at least one of the synthetic peptides comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-224, 502-508, 515-520, 524, 525, 527, and 529.

In some embodiments, the presently disclosed compositions at least one of the synthetic peptides comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, 502, and 509-529.

In some embodiments, the presently disclosed compositions at least one of the synthetic target peptides is a phosphopeptide or a phosphopeptide mimetic.

In some embodiments, the presently disclosed compositions at least one of the synthetic target peptides is a phosphopeptide mimetic comprising a mimetic of phosphoserine, phosphothreonine, or phosphotyrosine.

In some embodiments, the presently disclosed compositions the phosphopeptide mimetic is a synthetic molecule in which a phosphorous atom is linked to the serine, threonine, or tyrosine amino acid residue through a carbon.

In some embodiments, the presently disclosed compositions the composition further comprises a tetanus peptide. In some embodiments, the tetanus peptide comprises an amino acid sequence that is at least 90%, 95%, or 100% identical to SEQ ID NO: 449 or SEQ ID NO: 450. In some embodiments, the tetanus peptide is about or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 natural or non-natural amino acids in length. In some embodiments, the tetanus peptide comprises an amino acid sequence that is at least 90% identical to a 10-25 amino acid subsequence of a wild type tetanus toxoid protein. In some embodiments, the tetanus peptide binds to one or more MHC Class II molecules when administered to a subject. In some embodiments, the tetanus peptide is modified so as to prevent formation of tetanus peptide secondary structures.

The presently disclosed subject matter also provides in some embodiments *in vitro* populations of dendritic cells comprising the presently disclosed compositions.

The presently disclosed subject matter also provides in some embodiments *in vitro* populations of $CD8^+$ T cells capable of being activated upon being brought into contact

with a population of dendritic cells, wherein the dendritic cells comprise a composition of the presently disclosed subject matter .

The presently disclosed subject matter also provides in some embodiments antibodies and antibody-like molecules that specifically bind to a complex of an MHC class I molecule and a peptide, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-448 and 502-529. In some embodiments, the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, 502, and 509-529. In some embodiments, the antibodies or antibody-like molecules are members of the immunoglobulin superfamily. In some embodiments, the antibodies or antibody-like molecules comprise one or more binding members selected from the group consisting an Fab, Fab', $F(ab')_2$, Fv, and a single-chain antibody.

In some embodiments, the antibodies or antibody-like molecules of the presently disclosed subject matter are conjugated to a therapeutic agent selected from the group consisting of an alkylating agent, an antimetabolite, a mitotic inhibitor, a taxoid, a vinca alkaloid, and an antibiotic. In some embodiments, an antibody or antibody-like molecule of the presently disclosed subject matter is a T cell receptor, optionally conjugated to a CD3 agonist.

The presently disclosed subject matter also provides in some embodiments *in vitro* populations of T cells transfected with a nucleic acid, optionally an mRNA, encoding a T cell receptor of the presently disclosed subject matter.

The presently disclosed subject matter also provides in some embodiments methods for treating and/or preventing cancer comprising administering to a subject in need thereof a therapeutically effective dose of a presently disclosed composition and/or a composition comprising at least one target peptide comprising an amino acid sequence as set forth in any of SEQ ID NOs: 1-448 and 502-529. In some embodiments, the cancer is HCC, and the at least one target peptide comprises an amino acid sequence as set forth in any of SEQ ID NOs: 1-448. In some embodiments, the at least one target peptide comprises an amino acid sequence as set forth in any of SEQ ID NOs: 4, 5, 10, 11, 15, 24,

32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, 502, and 509-529. In some embodiments, the at least one target peptide comprises an amino acid sequence as set forth in any of SEQ ID NOs: 16, 36, 49, 54, 81, 105, 111, 137, 139, 140, 149, 156, 159, 166, 182, 191, 193, 196, 205, 216, 242, 249, 252, 257, 259, 262, 268, 269, 271, 289, 294, 296, 374, 376, 380, 381, 385, 428, and 502-508.

The presently disclosed subject matter also provides in some embodiments methods of treating and/or preventing hepatocellular carcinoma (HCC) and/or esophageal cancer comprising administering to a subject in need thereof a therapeutically effective dose of a presently disclosed composition or a composition comprising at least one target peptide in combination with a pharmaceutically acceptable carrier.

The presently disclosed subject matter also provides in some embodiments methods for treating and/or preventing cancer, optionally hepatocellular carcinoma (HCC) and/or esophageal cancer. In some embodiments, the presently disclosed methods comprise administering to a subject in need thereof a therapeutically effective dose of the presently disclosed $CD8^+$ T cells in combination with a pharmaceutically acceptable carrier.

The presently disclosed subject matter also provides in some embodiments methods for treating and/or preventing cancer, optionally hepatocellular carcinoma (HCC) and/or esophageal cancer, comprising administering to a subject in need thereof a presently disclosed *in vitro* population of dendritic cells in combination with a pharmaceutically acceptable carrier.

The presently disclosed subject matter also provides in some embodiments methods for treating and/or preventing hepatocellular carcinoma (HCC) and/or esophageal cancer, comprising administering to a subject in need thereof a presently disclosed population of $CD8^+$ T cells in combination with a pharmaceutically acceptable carrier.

The presently disclosed subject matter also provides in some embodiments methods for making a cancer vaccine, optionally a cancer vaccine for use in treating and/or preventing hepatocellular carcinoma (HCC) and/or esophageal cancer. In some embodiments, the presently disclosed methods comprise combining a presently disclosed

composition with an the adjuvant selected from the group consisting of montanide ISA-51, QS-21, a tetanus helper peptide, GM-CSF, cyclophosamide, bacillus Calmette-Guerin (BCG), corynbacterium parvum, levamisole, azimezone, isoprinisone, dinitrochlorobenezene (DNCB), keyhole limpet hemocyanin (KLH), complete Freunds adjuvant, in complete Freunds adjuvant, a mineral gel, aluminum hydroxide (Alum), lysolecithin, a pluronic polyol, a polyanion, an adjuvant peptide, an oil emulsion, dinitrophenol, and diphtheria toxin (DT), or any combination thereof and a pharmaceutically acceptable carrier; and placing the composition, adjuvant, and pharmaceutical carrier into a container, optionally into a syringe.

The presently disclosed subject matter also provides in some embodiments methods for screening target peptides for inclusion in the presently disclosed immunotherapy compositions or for use in the presently disclosed methods for using the presently disclosed compositions. In some embodiments, the methods comprise administering the target peptide to a human; determining whether the target peptide is capable of inducing a target peptide-specific memory T cell response in the human; and selecting the target peptide for inclusion in an immunotherapy composition if the target peptide elicits a memory T cell response in the human.

The presently disclosed subject matter also provides in some embodiments methods for determining a prognosis of a hepatocellular carcinoma (HCC) patient and/or an esophageal cancer patient, the methods comprising administering to the patient a target peptide comprising an amino acid sequence as set forth in any of SEQ ID NOs: 1-448 and 502-529, wherein the target peptide is associated with the patient's HCC and/or esophageal cancer; determining whether the target peptide is capable of inducing a target peptide-specific memory T cell response in the patient; and determining that the patient has a better prognosis if the patient mounts a memory T cell response to the target peptide than if the patient did not mount a memory T cell response to the target peptide. In some embodiments, the target peptide comprises an amino acid sequence as set forth in any of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, and 509-529.

The presently disclosed subject matter also provides in some embodiments kits comprising at least one target peptide composition comprising at least one target peptide comprising an amino acid sequence as set forth in any of SEQ ID NOs: 1-448 and 502-529 and a cytokine and/or an adjuvant. In some embodiments, the target peptide comprises an amino acid sequence as set forth in any of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, and 509-529. In some embodiments, the presently disclosed kits comprise at least 2, 3, 4, or 5 target peptide compositions. In some embodiments, the at least one target peptide composition is one of the compositions of disclosed herein. In some embodiments, the cytokine is selected from the group consisting of a transforming growth factor (TGF), optionally TGF-alpha and/or TGF-beta; insulin-like growth factor-I; insulin-like growth factor-II; erythropoietin (EPO); an osteoinductive factor; an interferon, optionally interferon-alpha, interferon-beta, and/or interferon-gamma; and a colony stimulating factor (CSF), optionally macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), and/or granulocyte-CSF (G-CSF). In some embodiments, the adjuvant is selected from the group consisting of montanide ISA-51, QS-21, a tetanus helper peptide, GM-CSF, cyclophosphamide, bacillus Calmette-Guerin (BCG), corynbacterium parvum, levamisole, azimezone, isoprinisone, dinitrochlorobenezene (DNCB), a keyhole limpet hemocyanin (KLH), complete Freund's adjuvant, incomplete Freund's adjuvant , a mineral gel, aluminum hydroxide, lysolecithin, a pluronic polyol, a polyanion, an adjuvant peptide, an oil emulsion, dinitrophenol, and diphtheria toxin (DT). In some embodiments, the cytokine is selected from the group consisting of a nerve growth factor, optionally nerve growth factor (NGF) beta; a platelet-growth factor; a transforming growth factor (TGF), optionally TGF-alpha and/or TGF-beta; insulin-like growth factor-I; insulin-like growth factor-II; erythropoietin (EPO); an osteoinductive factor; an interferon, optionally interferon-α, interferon-β, and/or interferon-γ; a colony stimulating factor (CSF), optionally macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), and/or granulocyte-CSF (G-CSF); an interleukin (IL), optionally IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-13, IL-14, IL-15, IL-16, IL-17, and/or IL-18; LIF; EPO; kit-ligand;

fms-related tyrosine kinase 3 (FLT-3; also called CD135); angiostatin; thrombospondin; endostatin; tumor necrosis factor; and lymphotoxin (LT).

In some embodiments, the presently disclosed kits further comprise at least one peptide derived from MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGE), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, β-HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, and TPS.

In some embodiments, the at least one target peptide comprises an amino acid sequence as set forth in any of SEQ ID NOs: 1-448 and 502-529. In some embodiments, the at least one target peptide is selected from the group consisting of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, 502, and 509-529. In some embodiments, the at least one target peptide is selected from the group consisting of SEQ ID NOs: 1-224, 502-508, 515-520, 524, 525, 527, and 528, and any combination thereof. In some embodiments, the at least one target peptide is selected from the group consisting of SEQ ID NOs: 502-508, and any combination thereof. In some embodiments, the at least one target peptide composition comprises one or more synthetic target peptides that specifically bind to an HLA molecule listed in Table 1 and/or that comprises an amino acid sequence at least 90% identical, optionally 100% identical, to one of the SEQ ID NOs: listed in Tables 2, 3, 5-7, and 14. In some embodiments, the kit comprises at least two synthetic target peptides, wherein the at least two synthetic target peptides are in separate containers.

Table 1

Anchor Residues for Different HLA Molecules

	Residue 1	Residue 2	Residue 3	Residue 7	Residue 9 or Last Residue
HLA A*0101		T, S	D, E		Y
HLA A*0201		L, M			V
HLA A*0301		L, M			K
HLA A*24		Y, W, M			L, F, W
HLA B*0702		P			L, M, V, F
HLA B*1508		P, A			Y
HLA B*2705	R	R			L, F, K, R, M
HLA B*4402		E			F, Y, W
HLA C*0501	Y	P, A	D		F, I, L, M, V
HLA C*0602	F, Y	R, Y	A, F, Y	K, Q, R	I, L, M, V

In some embodiments, the presently disclosed kits further comprise instructions related to determining whether the at least one synthetic target peptide of the at least one synthetic target peptide composition is capable of inducing a T cell memory response that is a T cell central memory response (Tcm) when the at least one synthetic target peptide composition is administered to a patient.

In some embodiments, the presently disclosed kits further comprise a tetanus peptide. In some embodiments, the tetanus peptide comprises an amino acid sequence that is at least 90%, 95%, or 100% identical to SEQ ID NO: 449 or SEQ ID NO: 450. In some embodiments, the tetanus peptide is about or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 natural or non-natural amino acids in length. In some embodiments, the tetanus peptide comprises an amino acid sequence that is at least 90% identical to a 10-25 amino acid subsequence of a wild type tetanus toxoid protein. In some embodiments, the tetanus peptide binds to one or more MHC Class II molecules when administered to a subject.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C present a summary of the characteristics of the first 250 MHC-I-pP analyzed and their presentation. Figure 1A is a bar graph showing that more different MHC-I-pP were presented per gram of tissue during progression of liver disease. Figure

1B is a bar graph showing that a greater diversity but not more MHC-I-pP were presented by each cell during the course of disease. Figure 1C is a bar graph of predicted HLA-binding of the first 250 identified HCC-specific MHC-I-pP. The most common represented types are HLA-A*0201, HLA-B*0702, HLA-B*2705, and HLA-C*07. In over ninety percent of the cases, the amino acid serine (S) was phosphorylated in HCC-specific MHC-I-pP, and the phosphate moiety was most often present at amino acid position 4 of the peptides. Abbreviations – AHL: adjacent "healthy" (*i.e.*, non-cirrhotic) liver; ACL: adjacent cirrhotic liver; HCC: hepatocellular carcinoma tissue; HepG2: HepG2 cell line; OEAC: esophageal cancer.

Figure 2 presents Boolean combination gates calculated and plotted as column graphs in order to assess the percentage of reactive T cells. Abbreviations – HD: healthy donor; HH: hereditary hemochromatosis patient; APC: antigen-presenting cell; IFNg-PE: phycoerythrin-labeled interferon gamma; CD107a: Cluster of Differentiation antigen 107a; IFNg: interferon gamma; TNFa: tumor necrosis factor alpha.

Figures 3A-3C present a summary of the characteristics of phosphopeptide-specific T cells in the blood compartment from patients with chronic liver disease. Figure 3A is a bar graph summarizing the results of the analysis of ppCTL by 7-day flow cytometry, which revealed that phosphopeptide-specific cells (pP) produced multiple cytokines and the similar amounts of cytotoxic markers in comparison to virus-specific T cells (viral). Figure 3B is a bar graph showing that ppCTLs resided in the memory compartment as determined by surface marker expression of CD45RA and CD27. As a control, the majority of unspecific T cells in PBMCs displayed a naive phenotype. Figure 3C is a plot showing that ppCTLs expressed higher amounts of CTLA-4, but not PD-1, on their surface in comparison to virus-specific T cells. Expanded ppCTLs recognized the phosphorylated embodiment of the peptide IMDRtPEKL (SEQ ID NO: 14 with Thr5 phosphorylated), but did not recognize the unphosphorylated counterpart IMDRTPEKL (SEQ ID NO: 14 with Thr5 non-phosphorylated), meaning that the expanded ppCTLs were phosphopeptide-specific rather than reactive toward the unphosphorylated counterpart peptide. Abbreviations – n.s.: not significant; viral: virus-specific T cell response; pP: ppCTL response; CD3: Cluster of Differentiation Antigen 3; CD107a: Cluster of Differentiation Antigen 107a; IFNg: interferon gamma; TNFa: tumor necrosis factor alpha; N: negative control (DMSO); EFF: effector T cells; MEM: memory T cells; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; PD-1: programmed cell death protein 1.

Figures 4A and 4B are graphs showing rapid expansion of liver-derived lymphocytes. Figure 4A is a graph showing that the rapid expansion protocol (REP) described in Dudley *et al.*, 2003 worked independently if the lymphocyte culture was initiated from "healthy" intrahepatic lymphocytes (DDL REP; open squares), cirrhotic intrahepatic lymphocytes (Cirrhotic IHL; open circles), or cancerous tumor-infiltrating lymphocytes (HCC TIL REP; black squares) tissue. Figure 4B is a graph showing that $CD8^+$ pre-selected cultures (black squares) expanded significantly faster than unselected cultures (open circles) in the first 14 days (d).

Figures 5A and 5B present the results of experiments that showed that ppCTLs were lost using REP but could be restored if lymphocyte cultures were expanded antigen-specifically. Figure 5A is a statistical summary of all positive ppCTL-responses comparing unspecific and specific expansion. No difference is observed for virus-specific T cells. Figure 5B is a Box and Whiskers plot of the data from Table 23 calculated with GraphPad (GraphPad Software, Inc., La Jolla, California, United States of America) showing that ppCTLs after expansion were functional, produced multiple cytokines, and were able to degranulate. The boxes extend from the 25th to 75th percentiles. The whiskers represent min and max values. Abbreviations – pP: phosphopeptide; n.s.: not significant; CD107a: Cluster of Differentiation antigen 107a; IFNg: interferon gamma; TNFa: tumor necrosis factor alpha.

Figure 6 is a LogoPlot depicting the residue frequency at each position of exemplary 9-mer HLA-*A02-phosphopeptides. HLA-A*2-associated phosphopeptides have unique characteristics that distinguish them from nonphosphorylated peptides. There was a strong preference for a positively charged amino acid at position 1, a leucine at position 2, the phosphopeptide at position 4, and a valine or leucine at position 9.

Figure 7 is an example of a typical analysis and graphical representation of a phosphopeptide-specific $CD8^+$ T cell response. Boolean combinatorial gates were calculated from an intracellular cytokine staining (ICS) experiment and the percentage of cytokine producing or degranulating T cells was assessed. In this case, PBMCs were reactive (>1% reactive cells) against the viral peptide NLVPMVATV (CMV, pp65; SEQ ID NO: 455) and MHC-I-pP AVVsPPALHNA (SEQ ID NO: 6) from Bromodomain-containing protein 4 (BRD4). In both cases (viral peptide and phosphopeptide), T cells responses were comparable in quantity and quality (polyfunctional cytokine production). Abbreviations – CD107a: Cluster of Differentiation Antigen 107a; IFNg: interferon

gamma; TNFa: tumor necrosis factor alpha; positive: positive control (PMA/Ionomycin); negative: negative control (DMSO).

Figure 8 is another example of a typical analysis and graphical representation of a phosphopeptide-specific $CD8^+$ T cell response showing an *ex vivo* $CD8^+$ T cell response against the phosphopeptide RVAsPTSGV (SEQ ID NO: 57) from insulin receptor substrate-2 (IRS2) after stimulation of PBMCs for 4 hours with the peptide. Abbreviations – DMSO: dimethyl sulfoxide; IRS2 (RVAsPTSGV): Insulin receptor substrate 2 phosphopeptide RVAsPTSGV (SEQ ID NO: 57); IFNg-PE: phycoerythrin-labeled interferon gamma; TNFa-PE-Cy7: TNFa labeled with phycoerythrin-Cyanin 5.1; IFNg: interferon gamma; TNFa: tumor necrosis factor alpha; RVAsPTSGV: phosphopeptide RVAsPTSGV (SEQ ID NO: 57); positive: positive control (PMA/Ionomycin); negative: negative control (DMSO).

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

A more complete understanding of the presently disclosed subject matter can be obtained by reference to the accompanying Sequence Listing, when considered in conjunction with the subsequent Detailed Description. The embodiments presented in the Sequence Listing are intended to be exemplary only and should not be construed as limiting the presently disclosed subject matter to the listed embodiments.

SEQ ID NOs: 1-448 are the amino acid sequences of exemplary MHC class I target peptides associated with HCC. Additional details with respect to optional post-translations modifications (*e.g.*, phosphorylation) of the amino acid sequences of SEQ ID NOs: 1-448 are provided in Tables 2-13 herein below.

SEQ ID NOs: 449 and 450 are the amino acid sequences of exemplary tetanus helper peptides.

SEQ ID NO: 451 is the amino acid sequence of a peptide from the cytomegalovirus (CMV; also referred to as human herpesvirus 5) phosphoprotein 65. It corresponds to amino acids 495-503 of Accession No. YP_081531.1 in the GENBANK® biosequence database.

SEQ ID NOs: 452-499 are exemplary peptides derived from various tumor-associated antigens (TAAs).

SEQ ID NO: 500 is the amino acid sequence of a Pan DR T helper epitopes (PADRE) peptide.

SEQ ID NO: 501 is the amino acid sequence of a peptide derived from the Epstein-Barr virus (EBV; also known as human herpesvirus 4) BMLF1 protein, which corresponds

to amino acids 259-267 of Accession No. YP_401660.1 in the GENBANK® biosequence database.

SEQ ID NOs: 502-508 are the amino acid sequences of exemplary MHC class I target peptides associated with esophageal cancer. Additional details with respect to optional post-translations modifications (*e.g.*, phosphorylation) of the amino acid sequences of SEQ ID NOs: 502-508 are provided in Table 14 herein below.

SEQ ID NOs: 509-529 are the amino acid sequences of additional exemplary MHC class I target peptides associated with HCC. Additional details with respect to optional post-translations modifications (*e.g.*, phosphorylation) of the amino acid sequences of SEQ ID NOs: 509-529 are provided in Tables 2, 3, 6, and 9 herein below.

DETAILED DESCRIPTION

I. General Considerations

Advanced hepatocellular carcinoma (HCC) and esophageal cancer are serious therapeutic challenges and novel approaches are urgently needed for the treatment of these conditions. The immune system can specifically identify and eliminate tumor cells on the basis of their expression of tumor-associated antigens (TAA). This process is referred to as tumor immune surveillance, whereby the immune system identifies cancerous and/or precancerous cells and eliminates them before they can cause harm (Corthay, 2014). Therefore, immunotherapy is considered a promising new treatment modality. The basis for every immunotherapeutic approach is the identification of specific targets, which distinguishes the malignant cells from healthy cells. Very few immunogenic TAA have been characterized so far in general and even less for HCC in particular, which is considered to be an immunogenic tumor (Prieto *et al.*, 2015).

During the course of chronic liver disease, for example, several mutations and epigenetic changes accumulate in the liver cells, which finally lead to a dysregulation of major signaling pathways that are important for malignant transformation (Whittaker *et al.*, 2010). Similar processes are likely to be occurring in cells that give rise to esophageal cancer. Therefore, deregulation of signaling pathways with altered and augmented phosphorylation of cellular proteins is a hallmark of tumorigenesis generally and malignant transformation in particular. Phosphoproteins involved in these signaling cascades can be degraded to phosphopeptides that are presented by major histocompatibility complex (MHC) class I and -II molecules and recognized by T cells (Zarling *et al.*, 2000; Zarling *et al.*, 2006; Cobbold *et al.*, 2013). The contributions of

phosphopeptide-specific T cells to immune surveillance in the development of liver cancer in chronic liver disease and in tumorigenesis leading to esophageal cancer are unclear.

It was hypothesized that phosphopeptides are presented by MHC molecules with increasing amounts on the surface of altered hepatocytes and esophageal cells with progression of liver disease towards HCC and tumorigenesis leading to esophageal cancer. It was further hypothesized that the immune system monitors the liver for malignant transformed hepatocytes and the esophagus for tumorigenic cells and clears those cells with the help of phosphopeptide-specific cytotoxic T lymphocytes (ppCTLs).

Therefore, MHC class I-associated phosphopeptides (MHC-I-pP) that are presented on the surface of HCC and cells involved with tumorigenesis leading to esophageal cancer were investigated using a mass spectrometry approach. In order to show the immunogenicity of these novel identified tumor antigens, the T cell responses to these newly identified phosphoantigens in healthy individuals, in patients with chronic liver diseases, and in patients with HCC were characterized. The quantity and quality of these tumor-specific T cell responses was correlated with the patients' clinical course and HCC tumor and esophageal cancer progression.

As such, disclosed herein is a set of 460 phosphopeptides presented to the immune system by class I MHC molecules derived from human hepatocellular carcinoma (HCC), some of which are also derived from esophageal cancer, and seven (7) phosphopeptides presented to the immune system by class I MHC molecules derived from esophageal cancer but not HCC. These peptides have at least the potential to (a) stimulate an immune response to the cancer; (b) function as immunotherapeutics in adoptive T-cell therapy or as vaccine; (c) function as targets for immunotherapy based on bispecific antibodies; (d) facilitate antibody recognition of the tumor boundaries in surgical pathology samples; and (e) act as biomarkers for early detection of the disease, although the presently disclosed subject matter is not limited to just these applications.

II. Definitions

While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter.

All technical and scientific terms used herein, unless otherwise defined below, are intended to have the same meaning as commonly understood by one of ordinary skill in the art. Mention of techniques employed herein are intended to refer to the techniques as commonly understood in the art, including variations on those techniques or substitutions

of equivalent techniques that would be apparent to one of skill in the art. While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter. Thus, unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of the presently disclosed subject matter. Although any compositions, methods, kits, and means for communicating information similar or equivalent to those described herein can be used to practice the presently disclosed subject matter, particular compositions, methods, kits, and means for communicating information are described herein. It is understood that the particular compositions, methods, kits, and means for communicating information described herein are exemplary only and the presently disclosed subject matter is not intended to be limited to just those embodiments.

Following long-standing patent law convention, the terms "a", "an", and "the" refer to "one or more" when used in this application, including the claims. Thus, in some embodiments the phrase "a peptide" refers to one or more peptides.

The term "about", as used herein to refer to a measurable value such as an amount of weight, time, dose (*e.g.*, therapeutic dose), etc., is meant to encompass in some embodiments variations of ±20%, in some embodiments ±10%, in some embodiments ±5%, in some embodiments ±1%, in some embodiments ±0.1%, in some embodiments ±0.5%, and in some embodiments ±0.01% from the specified amount, as such variations are appropriate to perform the disclosed methods.

As used herein, the term "and/or" when used in the context of a list of entities, refers to the entities being present singly or in any and every possible combination and subcombination. Thus, for example, the phrase "A, B, C, and/or D" includes A, B, C, and D individually, but also includes any and all combinations and subcombinations of A, B, C, and D. It is further understood that for each instance wherein multiple possible options are listed for a given element (*i.e.*, for all "Markush Groups" and similar listings of optional components for any element), in some embodiments the optional components can be present singly or in any combination or subcombination of the optional components. It is implicit in these forms of lists that each and every combination and subcombination is envisioned and that each such combination or subcombination has not been listed simply merely for convenience. Additionally, it is further understood that all recitations of "or" are to be interpreted as "and/or" unless the context clearly requires that listed components

be considered only in the alternative (*e.g.*, if the components would be mutually exclusive in a given context and/or could not be employed in combination with each other).

As used herein, the phrase "amino acid sequence as set forth in any of SEQ ID NOs: [A]-[B]" refers to any amino acid sequence that is disclosed in any one or more of SEQ ID NOs: A-B. In some embodiments, the amino acid sequence is any amino acid sequence that is disclosed in any of the SEQ ID NOs. that are present in the Sequence Listing. In some embodiments, the phrase refers to the full length sequence of any amino acid sequence that is disclosed in any of the SEQ ID NOs. that are present in the Sequence Listing, such that an "amino acid sequence as set forth in any of SEQ ID NOs: [A]-[B]" refers to the full length sequence of any of the sequences disclosed in the Sequence Listing. By way of example and not limitation, in some embodiments an "amino acid sequence as set forth in any of SEQ ID NOs: 1-448 and 502-529" refers to the full length amino acid sequence disclosed in any of SEQ ID NOs: 1-448 and 502-529 and not to a subsequence of any of SEQ ID NOs: 1-448 and 502-529.

The presently disclosed subject matter relates in some embodiments to post-translationally-modified immunogenic therapeutic target peptides, *e.g.*, phosphopeptides, for use in immunotherapy and diagnostic methods of using the target peptides, as well as methods of selecting the same to make compositions for immunotherapy, *e.g.*, in vaccines and/or in compositions useful in adaptive cell transfer.

III. Target Peptides

The presently disclosed subject matter relates in some embodiments to immunogenic therapeutic target peptides for use in immunotherapy and diagnostic methods of using the target peptides, as well as methods of selecting the same to make compositions for immunotherapy, *e.g.*, in vaccines and/or in compositions useful in adaptive cell transfer. In some embodiments, the target peptides of the presently disclosed subject matter are post-translationally modified by being provided with a phosphate group, (*i.e.*, "phosphopeptides"). In some embodiments, the target peptides of the presently disclosed subject matter are modified by having an oxidized methionine.

The target peptides of the presently disclosed subject matter are in some embodiments not the entire proteins from which they are derived. They are in some embodiments from 6 to 50 contiguous amino acid residues of the native human protein. They can in some embodiments contain exactly, about, or at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids. The peptides of the

presently disclosed subject matter can also in some embodiments have a length that falls in the ranges of 6-10, 9-12, 10-13, 11-14, 12-15, 15-20, 20-25, 25-30, 30-35, 35-40, and 45-50 amino acids. Exactly, about, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or more of the amino acid residues within the recited sequence of a target peptide can phosphorylated.

Target peptides can be modified and analogs (using for example, beta-amino acids, L-amino acids, N-methylated amino acids, amidated amino acids, non-natural amino acids, retro inverse peptides, peptoids, PNA, halogenated amino acids) can be synthesized that retain their ability to stimulate a particular immune response, but which also gain one or more beneficial features, such as those described below. Thus, particular target peptides can, for example, have use for treating and vaccinating against multiple cancer types.

In some embodiments, substitutions can be made in the target peptides at residues known to interact with the MHC molecule. Such substitutions can in some embodiments have the effect of increasing the binding affinity of the target peptides for the MHC molecule and can also increase the half-life of the target peptide-MHC complex, the consequence of which is that the analog is in some embodiments a more potent stimulator of an immune response than is the original peptide.

Additionally, the substitutions can in some embodiments have no effect on the immunogenicity of the target peptide *per se*, but rather can prolong its biological half-life or prevent it from undergoing spontaneous alterations which might otherwise negatively impact on the immunogenicity of the peptide.

The target peptides disclosed herein can in some embodiments have differing levels of immunogenicity, MHC binding and ability to elicit CTL responses against cells displaying a native target peptide, *e.g.*, on the surface of a tumor cell.

The amino acid sequences of the target peptides can in some embodiments be modified such that immunogenicity and/or binding is enhanced. In some embodiments, the modified target peptide binds an MHC class I molecule about or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, 110%, 125%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 350%, 375%, 400%, 450%, 500%, 600%, 700%, 800%, 1000%, or more tightly than its native (unmodified) counterpart.

However, given the exquisite sensitivity of the T-cell receptor, it cannot be foreseen whether such enhanced binding and/or immunogenicity will render a modified target peptide still capable of inducing an activated CTL that will cross react with the native target peptide being displayed on the surface of a tumor. Indeed, it is disclosed

herein that the binding affinity of a target peptide does not predict its functional ability to elicit a T cell response.

Target peptides of the presently disclosed subject matter can in some embodiments be mixed together to form a cocktail. The target peptides can in some embodiments be in an admixture, or they can in some embodiments be linked together in a concatamer as a single molecule. Linkers between individual target peptides can in some embodiments be used; these can, for example, in some embodiments be formed by any 10 to 20 amino acid residues. The linkers can in some embodiments be random sequences, or they can in some embodiments be optimized for degradation by dendritic cells.

In certain specified positions, a native amino acid residue in a native human protein can in some embodiments be altered to enhance the binding to the MHC class I molecule. These can occur in "anchor" positions of the target peptides, often in positions 1, 2, 3, 9, or 10. Valine (V), alanine (A), lysine (K), leucine (L), isoleucine (I), tyrosine (Y), arginine (R), phenylalanine (F), proline (P), glutamic acid (E), glutamine (Q), threonine (T), serine (S), aspartic acid (D), tryptophan (W), and methionine (M) can also be used in some embodiments as improved anchoring residues. Anchor residues for different HLA molecules are listed below. Anchor residues for HLA molecules are listed in Table 1.

In some embodiments, the immunogenicity of a target peptide is measured using transgenic mice expressing human MHC class I genes. For example, "ADD Tg mice" express an interspecies hybrid class I MHC gene, AAD, which contains the alpha-1 and alpha-2 domains of the human HLA-A2.1 gene and the alpha-3 transmembrane and cytoplasmic domains of the mouse H-2Dd gene, under the direction of the human HLA-A2.1 promoter. Immunodetection of the HLA-A2.1 recombinant transgene established that expression was at equivalent levels to endogenous mouse class I molecules. The mouse alpha-3 domain expression enhances the immune response in this system. Compared to unmodified HLA-A2.1, the chimeric HLA-A2.1/H2-Dd MHC Class I molecule mediates efficient positive selection of mouse T cells to provide a more complete T cell repertoire capable of recognizing peptides presented by HLA-A2.1 Class I molecules. The peptide epitopes presented and recognized by mouse T cells in the context of the HLA-A2.1/H2-Dd class I molecule are the same as those presented in HLA-A2.1^{+} humans. This transgenic strain facilitates the modeling of human T cell immune responses to HLA-A2 presented antigens, and identification of those antigens. This transgenic strain is a

preclinical model for design and testing of vaccines for infectious diseases or cancer therapy involving optimal stimulation of $CD8^+$ cytolytic T cells.

In some embodiments, the immunogenicity of a modified target peptide is determined by the degree of Interferon gamma and/or TNF-α production of T-cells from ADD Tg mice immunized with the target peptide, *e.g.*, by immunization with target peptide pulsed bone marrow derived dendritic cells.

In some embodiments, the modified target peptides are about or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, 110%, 125%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 350%, 375%, 400%, 450%, 500%, 600%, 700%, 800%, 1000%, 1500%, 2000%, 2500%, 3000%, 4000%, 5000%, or more immunogenic, *e.g.*, in terms of numbers of Interferon gamma and/or TNF-alpha positive (*i.e.*, "activated") T-cells relative to numbers elicited by native target peptides in ADD Tg mice immunized with target peptides pulsed bone marrow derived dendritic cells. In some embodiments, the modified target peptides are able to elicit $CD8^+$ T cells which are cross-reactive with the modified and the native target peptide in general and when such modified and native target peptides are complexed with MHC class I molecules in particular. In some embodiments, the $CD8^+$ T cells which are cross-reactive with the modified and the native target peptides are able to reduce tumor size by about or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, or 99% in a NOD/SCID/IL-2Rγc$^{-/-}$ knock out mouse (which has been provided transgenic T cells specific form an immune competent donor) relative to IL-2 treatment without such cross-reactive $CD8^+$ T cells.

The term "capable of inducing a target peptide-specific memory T cell response in a patient" as used herein relates to eliciting a response from memory T cells (also referred to as "antigen-experienced T cell") which are a subset of infection- and cancer-fighting T cells that have previously encountered and responded to their cognate antigen. Such T cells can recognize foreign invaders, such as bacteria or viruses, as well as cancer cells. Memory T cells have become "experienced" by having encountered antigen during a prior infection, encounter with cancer, or previous vaccination. At a second encounter with the cognate antigen, *e.g.*, by way of an initial inoculation with a target peptide of the presently disclosed subject matter, memory T cells can reproduce to mount a faster and stronger immune response than the first time the immune system responded to the invader (*e.g.*, through the body's own consciously unperceived recognition of a target peptide being associated with diseased tissue). This behavior can be assayed in T lymphocyte proliferation assays, which can reveal exposure to specific antigens. Memory T cells

comprise two subtypes: central memory T cells (T_{CM} cells) and effector memory T cells (T_{EM} cells). Memory cells can be either $CD4^+$ or $CD8^+$. Memory T cells typically express the cell surface protein CD45RO. Central memory T_{CM} cells generally express L-selectin and CCR7, they secrete IL-2, but not IFNγ or IL-4. Effector memory T_{EM} cells, however, generally do not express L-selectin or CCR7 but produce effector cytokines like IFNγ and IL-4.

A memory T cell response generally results in the proliferation of memory T cell and/or the upregulation or increased secretion of the factors such as CD45RO, L-selectin, CCR7, IL-2, IFNγ, CD45RA, CD27, and/or IL-4. In some embodiments, the target peptides of the presently disclosed subject matter are capable of inducing a T_{CM} cell response associated with L-selectin, CCR7, IL-2 (but not IFNγ or IL-4) expression and/secretion (*see e.g.*, Hamann *et al.*, 1997). In some embodiments, a T_{CM} cell response is associated with an at least or about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, 95%, 97%, 98%, 99%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 1500%, 2000%, or more increase in T cell CD45RO/RA, L-selectin, CCR7, or IL-2 expression and/secretion.

In some embodiments, the target peptides of the presently disclosed subject matter are capable of inducing a $CD8^+$ T_{CM} cell response in a patient the first time that patient is provided the composition including the selected target peptides. As such, the target peptides of the presently disclosed subject matter can in some embodiments be referred to as "neo-antigens". Although target peptides might be considered "self" for being derived from self-tissue, they generally are only found on the surface of cells with a dysregulated metabolism, *e.g.*, aberrant phosphorylation, they are likely never presented to immature T cells in the thymus. As such, these "self" antigens act are neo-antigens because they are nevertheless capable of eliciting an immune response.

In some embodiments, about or at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, 95%, 97%, 98%, or 99% of T cells activated by particular target peptide in a particular patient sample are T_{CM} cells. In some embodiments, a patient sample is taken exactly, about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more days after an initial exposure to a particular target peptide and then assayed for target peptide specific activated T cells and the proportion of T_{CM} cells thereof. In some embodiments, the compositions of the presently disclosed subject matter are able to elicit a $CD8^+$ T_{CM}

cell response in at least or about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, 95%, 97%, 98%, 99%, or 100% of patients and/or healthy volunteers. In some embodiments, the compositions of the presently disclosed subject matter are able to elicit a $CD8^+$ T_{CM} cell response in a patient about or at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, 95%, 97%, 98%, 99%, or 100% of patients and/or healthy volunteers specific to all or at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 target peptides in the composition. In some embodiments, the aforementioned T cell activation tests are done by ELISpot assay.

III. Phosphopeptides

In some embodiments, the target peptides of the presently disclosed subject matter are post-translationally-modified by being provided with a phosphate group (referred to herein as "phosphopeptides"). The term "phosphopeptides" includes MHC class I-specific phosphopeptides. Exemplary MHC class I phosphopeptides of the presently disclosed subject matter that are associated in some embodiments with hepatocellular carcinoma are set forth in Tables 2-14. The amino acid sequences of these phosphopeptides are set forth in SEQ ID NOs: 1-448 and 502-529. In Tables 2-14, phosphoserine, phosphothreonine, and phosphotyrosine residues are indicated by "s", "t", and "y", respectively. Oxidized methionine residues are indicated by "m". "Gene Name" refers to the name of the Gene as set forth in the UniProt biosequence database. A lowercase "c" in a peptide sequence indicates that in some embodiments the cysteine is present in a cysteine-cysteine disulfide bond at the surface of a cell and, in some embodiments, is presented to the immune system as such.

Table 2

Exemplary Class I MHC Phosphopeptides on HCC that are Specific for HLA-A*0201

SEQ ID NO.	Sequence[#]	Start	Stop	UniProt Acc. No.	Gene Name[%]
1	AIMRsPQMV	187	195	P35222	CTNNB1
2	ALDsGASLLHL	482	492	P57078	RIPK4
3	ALGNtPPFL	111	119	Q7Z739	YTHDF3
4	ALMGsPQLV	178	186	P14923	JUP
5	ALMGsPQLVAA	178	188	P14923	JUP
6	AVVsPPALHNA	905	915	O60885	BRD4
7	DLKRRsmSI	175	183	Q96N67	DOCK7

8	ELFSsPPAV	953	960	O94916	NFAT5
9	FLDtPIAKV	320	328	Q969G9	NKD1
10	GIDsPSSSV	77	85	Q5JSP0	FGD3
11	GLDsGFHSV	297	305	O75427	LRCH4
12	GLIsPVWGA	50	58	Q76N32	CEP68
13	GLLDsPTSI	218	226	Q07352	ZFP36L1
14	IMDRtPEKL	126	134	O75815	BCAR3
15	KAFsPVR	2	8	Q02363	ID2
16	KAFsPVRSV	2	10	Q02363	ID2
17	KIAsEIAQL	541	549	Q8WXE0	CASKIN2
18	KIGsIIFQV	1223	1231	Q460N5	PARP14
19	KLAsPELERL	70	79	P05412	JUN
20	KLDsPRVTV	38	46	D3DUF1	FAM86A
21	KLFPDtPLAL	587	596	Q12906	ILF3
22	KLIDIVsSQKV	461	471	O14757	CHEK1
23	KLIDRTEsL	197	205	P33241	LSP1
24	KLMsDVEDV	1940	1948	Q9NSI6	BRWD1
25	KLMsPKADVKL	44	54	Q86T90	KIAA1328
26	KQDsLVINL	647	655	Q9Y5B9	SUPT16H
27	KTMsGTFLL	592	600	P52630	STAT2
28	KVAsLLHQV	330	338	Q8NFZ5	TNIP2
29	LMFsPVTSL	887	895	Q9C0A6	SETD5
30	RASsLSITV	839	847	Q6ZS17	FAM65A
31	RLAsASRAL	?	?	Unknown	Unknown
32	RLAsLQSEV	?	?	Unknown	Unknown
33	RLAsYLDKV	90	98	P08727	KRT19
34	RLAsYLDRV	90	98	P05783	KRT18
35	RLDsYVR	129	135	Q9Y5R8	TRAPPC1
36	RLDsYVRSL	129	137	Q9Y5R8	TRAPPC1
37	RLFsKEL	30	36	Q15543	TAF13
38	RLFsKELR	30	37	Q15543	TAF13
39	RLFsKELRC	30	38	Q15543	TAF13

40	RLLsDLEEL	245	253	Q8IWP9	CCDC28A
41	RLLsTDAEAV	168	177	Q15545	TAF7
42	RLSDtPPLL	205	213	P20337	RAB3B
43	RLSsPLHFV	400	408	Q8NC44	FAM134A
44	RMYsFDDVL	802	810	Q8WWI1	LMO7
45	RQAsIELPSM	249	258	P33241	LSP1
46	RQAsIELPSMAV	249	260	P33241	LSP1
47	RQAsLSISV	526	534	Q9BZL6	PRKD2
48	RQDsTPGKVFL	61	71	P13056	NR2C1
49	RQIsQDVKL	165	173	Q01433	AMPD2
50	RQLsALHRA	31	39	P61313	RPL15
51	RQLsSGVSEI	79	88	P04792	HSPB1
52	RSLsESYEL	104	112	Q6DN90	IQSEC1
53	RSLsQELVGV	333	342	Q5VUA4	ZNF318
54	RTFsPTYGL	426	434	O15061	SYNM
55	RTLsHISEA	450	458	Q6ZS17	FAM65A
56	RTYsGPMNKV	53	62	Q8WVV4	POF1B
57	RVAsPTSGV	1097	1105	Q9Y4H2	IRS2
58	SImsPEIQL	154	162	Q96RK0	CIC
59	SISsMEVNV	149	157	Q9BQY9	DBNDD2
60	SISStPPAV	260	268	Q9H8Y8	GORASP2
61	SLFGGsVKL	103	111	Q8WUM4	PDCD6IP
62	SLFsGDEENA	22	31	Q53EL6	PDCD4
63	SLFsPQNTL	973	981	Q5VT52	RPRD2
64	SLFsSEESNL	403	412	P04004	VTN
65	SLFsSEESNLGA	30	38	Q15543	TAF13
66	SLHDIQLsL	694	702	Q9H7U1	CCSER2
67	SLQPRSHsV	448	456	Q9Y2H5	PLEKHA6
68	SLQsLETSV	1233	1241	P23634	ATP2B4
69	SMSsLSREV	2117	2125	O15027	SEC16A
70	SMTRsPPRV	248	256	Q9BRL6	SRSF8
71	SVKPRRTsL	766	774	P15822	HIVEP1

72	TVFsPTLPAA	375	384	Q7Z2W4	ZC3HAV1
73	VLFSsPPQM	67	75	P33991	MCM4
74	VLLsPVPEL	552	560	Q9H1A4	ANAPC1
75	VLYsPQMAL	372	380	O60502	MGEA5
76	VMIGsPKKV	1437	1445	Q68CZ2	TNS3
77	yLQSRYYRA	359	367	Q9H422	HIPK3
510	AMPGsPVEV	39	47	O43439	CBFA2T2
512	KVLsSLVTL	17	25	E7ENL8	ARHGEF7
513	KVYsSSEFL	39	47	V9GYV0	MAST3
514	RASsDIVSL	120	128	V9GZ26	FAM110A
514	RASsDIVsL	120	128	V9GZ26	FAM110A
521	RTYsGPMNK	53	61	Q8WVV4	POF1B

Table 3

Exemplary Class I MHC Phosphopeptides on HCC that are Specific for HLA-B*0702

SEQ ID NO.	Sequence[#]	Start	Stop	UniProt Acc. No.	Gene Name
78	APDsPRAFL	?	?	Unknown	Unknown
79	APRKGsFSAL	5	14	Q13619	CUL4A
80	APRNGsGVAL	549	558	Q7L9B9	EEPD1
81	APRRYsSSL	697	705	Q68EM7	ARHGAP17
82	APRsPPPSRP	8	17	Q9NSA8	SOCS-1
83	APSLFHLNtL	1230	1239	Q96QB1	DLC1
84	APSSARAsPLL	?	?	Unknown	Unknown
85	FPLDsPKTLVL	2071	2081	Q5VUA4	ZNF318
86	FPRRHsVTL	49	57	Q07352	ZFP36L1
87	FRGRYRsPY	91	99	Q14498	RBM39
88	FRKsMVEHY	97	106	Q14088	RAB33A
89	GPPYQRRGsL	359	368	P41161	ETV5
90	GPRPGsPSAL	266	275	Q9UJJ7	RPUSD1
91	GPRSAsLL	51	60	Q9Y4H4	GPSM3
92	GPRSAsLLSL	51	60	Q9Y4H4	GPSM3
93	GPRSAsLLsL	51	60	Q9Y4H4	GPSM3

94	GPRsPKAPP	71	79	Q6PJ34	ARHGAP4
95	HPKRSVsL	160	167	O60238	BNIP3L
96	HRYsTPHAF	230	238	P04049	RAF1
97	KPAsPKFIVTL	512	522	Q6PJT7	ZC3H14
98	KPPYRSHsL	442	450	Q96GE4	CEP95
99	KPRPLsMDL	279	287	Q9BY89	KIAA1671
100	KPRPPPLsP	328	336	Q8NFL1	TRIP10
101	KPRRFsRsL	209	217	Q7L4I2	RSRC2
101	KPRRFsRSL	209	217	Q7L4I2	RSRC2
102	KPRsPFSKI	185	193	Q9BXF6	RAB11FIP5
103	KPRsPPRAL	249	257	Q86TG7	PEG10
104	KPRsPPRALVL	249	259	Q86TG7	PEG10
105	KPRsPVVEL	667	675	P25098	GRK2
106	KPSsPRGSL	134	142	Q96IF1	AJUBA
107	KPSsPRGSLL	134	143	Q96IF1	AJUBA
108	KPVsPKSGTL	246	255	Q14155	ARHGEF7
109	KPYsPLASL	70	78	Q13469	NFATC2
110	KRAsGQAFEL	13	22	P16949	STMN1
111	LPAsPRARL	443	451	Q3KQU3	MAP7D1
112	LPIFSRLsI	483	491	P47974	ZFP36L2
113	LPKGLSAsL	541	549	Q6PKG0	LARP1
113	LPKGLsASL	541	549	Q6PKG0	LARP1
114	LPRGsSPSVL	105	114	Q9GZN2	TGIF2
115	LPRPAsPAL	2247	2255	P78559	MAP1A
116	LPRSSsMAA	361	369	Q9UQB8	BAIAP2
117	LPRSSsMAAGL	361	371	Q9UQB8	BAIAP2
118	MPRQPsATRL	134	143	Q6NZ67	MZT2B
119	QPRtPSPLVL	172	181	P33241	LSP1
120	RARGIsPIVF	303	312	Q96MU7	YTHDC1
121	RKLsVILIL	3	11	Q13433	SLC39A6
122	RLLsPQQPAL	177	186	Q14814	MEF2D
123	RPAFFsPSL	299	307	Q6ICG6	KIAA0930

124	RPAKsMDSL	323	331	Q7Z6I6	ARHGAP30
125	RPAsAGAmL	198	206	Q14814	MEF2D
126	RPAsPAAKL	512	520	Q9P2N6	KANSL3
127	RPAsPEPEL	?	?	Unknown	Unknown
128	RPAsPGPSL	646	654	Q8IY33	MICALL2
129	RPAsPQRAQL	?	?	Unknown	Unknown
130	RPAsPSLQL	277	285	Q8WUF5	PPP1R13L
131	RPAsPSLQLL	277	286	Q8WUF5	PPP1R13L
132	RPAsYKKKSML	764	774	P16234	PDGFRA
133	RPDsPTRPTL	1646	1655	Q7RTP6	MICAL3
134	RPDsRLGKTEL	1225	1235	Q9BYW2	SETD2
135	RPDVAKRLsL	282	291	O75815	BCAR3
136	RPFHGISTVsL	1417	1427	Q5VZ89	DENND4C
137	RPFsPREAL	742	750	Q86V48	LUZP1
138	RPGsRQAGL	175	184	Q96JY6	PDLIM2
139	RPIsPGLSY	364	372	Q16204	CCDC6
140	RPIsPPHTY	1303	1311	Q9Y6N7	ROBO1
141	RPIsPRIGAL	93	102	Q9Y6I3	EPN1
142	RPKLSsPAL	15	23	Q09472	EP300
143	RPKsNIVLL	222	230	P11836	MS4A1
144	RPKsPLSKM	1576	1584	Q9HCD6	TANC2
145	RPKsVDFDSL	455	464	Q9Y5K6	CD2AP
146	RPKtPPVVI	245	253	Q96A49	SYAP1
147	RPLsLLLAL	12	20	P78504	JAG1
148	RPLsVVYVL	43	51	O95382	MAP3K6
149	RPMsESPHM	280	288	Q07352	ZFP36L1
150	RPNsPSPTAL	185	194	Q9UKI8	TLK1
151	RPPsPGPVL	934	942	Q12770	SCAP
152	RPQRAtSNVF	14	23	P24844	MYL9
153	RPRAAtVV	333	340	P10644	PRKAR1A
154	RPRAAtVVA	333	341	P10644	PRKAR1A
155	RPRANsGGVDL	1162	1172	Q92766	RREB1

156	RPRARsVDAL	488	497	Q86X29	LSR
157	RPRDtRRISL	1862	1871	Q92508	PIEZO1
158	RPRGsESLL	?	?	Unknown	Unknown
159	RPRGsQSLL	1040	1048	P21860	ERBB3
160	RPRIPsPIGF	582	591	Q9NRA8	EIF4ENIF1
161	RPRPAsSPAL	266	275	A8MQ27	NEURL1B
162	RPRPHsAPSL	108	117	Q5JXC2	MIIP
163	RPRPSsAHVGL	958	961	Q8TF72	SHROOM3
164	RPRPsSVL	192	199	Q9NTK1	DEPP
165	RPRPsSVLRTL	?	?	Unknown	Unknown
166	RPRPVsPSSL	430	439	P57059	SIK1
167	RPRPVsPSSLL	430	440	P57059	SIK1
168	RPRsAVEQL	882	890	Q9HAU0	PLEKHA5
169	RPRsAVLL	1873	1880	Q12802	AKAP13
170	RPRsISVEEF	1143	1152	Q7Z333	SETX
171	RPRsLEVTI	239	247	O15553	MEFV
172	RPRSLsSPTVTL	443	454	Q96PU5	NEDD4L
173	RPRsMTVSA	457	465	O43312	MTSS1
174	RPRsMVRSF	1628	1636	Q14185	DOCK1
175	RPRsPAARL	111	119	Q9P2Y4	ZNF219
176	RPRsPNMQDL	214	223	Q6T310	RASL11A
177	RPRsPPGGP	573	581	Q86UZ6	ZBTB46
178	RPRsPPPRAP	499	508	O43900	PRICKLE3
179	RPRsPPSSP	41	49	P27815	PDE4A
180	RPRsPRENSI	689	698	Q99700	ATXN2
181	RPRsPRPPP	?	?	Unknown	Unknown
182	RPRsPRQNSI	689	698	Q99700	ATXN2
183	RPRSPsPIS	1015	1023	P41594	GRM5
184	RPRsPTGPSNSF	219	230	Q96I25	RBM17
185	RPRsPTGPSNSFL	219	231	Q96I25	RBM17
186	RPRsPWGKL	104	112	O43236	SEPT4
187	RPRsQYNTKL	494	503	Q7Z6B7	SRGAP1

188	RPRtPLRSL	?	?	Unknown	Unknown
189	RPSsLPDL	635	642	Q8NFD5	ARID1B
190	RPSsPALYF	261	269	Q9Y3Q8	TSC22D4
191	RPTsFADEL	285	293	Q9Y4E1	WASHC2C
192	RPTsRLNRL	860	868	Q15788	NCOA1
193	RPVsPFQEL	?	?	Unknown	Unknown
194	RPVsPGKDI	2115	2123	P31629	HIVEP2
195	RPVSPsSLL	432	440	P57059	SIK1
196	RPVsTDFAQY	666	675	O14639	ABLIM1
197	RPVtPVSDL	63	71	Q13118	KLF10
198	RPWsNSRGL	71	79	Q9NRR8	CDC42SE1
199	RPWsPAVSA	380	388	P12755	SKI
200	RPYsPPFFSL	187	196	Q9NYF3	FAM53C
201	RPYsQVNVL	165	173	P46939	UTRN
202	RTRsPSPTL	515	523	Q86UU1	PHLDB1
203	RVRKLPsTTL	726	735	Q15418	RPS6KA1
204	SPAsPKISL	493	501	Q8WWM7	ATXN2L
205	SPFKRQLsL	288	296	B7Z5W0	N/A
206	SPFLsKRSL	334	342	Q9NYV4	CDK12
207	SPGLARKRsL	851	860	Q9H2Y7	ZNF106
208	SPKsPGLKA	105	113	Q6JBY9	RCSD1
209	SPRERsPAL	243	251	Q9Y2W1	THRAP3
210	SPRGEASsL	167	175	Q8IY57	YAF2
210	SPRGEAsSL	167	175	Q8IY57	YAF2
211	SPRsPGRSL	?	?	Unknown	Unknown
212	SPRsPSGLR	1449	1457	P49815	TSC2
213	SPRSPsTTYL	772	781	Q13111	CHAF1A
214	SPSsPSVRRQL	1988	1998	O75179	ANKRD17
215	TPMKKHLsL	423	431	Q8IXS8	FAM126B
216	TPRsPPLGL	755	763	Q16584	MAP3K11
217	TPRsPPLGLI	755	764	Q16584	MAP3K11
218	VAKRLsL	285	291	O75815	BCAR3

219	VPRPERRsSL	668	677	Q6UWJ1	TMCO3
220	VPRsPKHAHSSSL	242	254	O95425	SVIL
221	VPTsPKSSL	1151	1159	Q70E73	RAPH1
222	YPDPHsPFAV	240	249	P41162	ETV3
223	YPGGRRsSL	1037	1045	P22897	MRC1
224	YPYEFsPVKM	121	130	Q6BEB4	SP5
515	RPAsEARAPGL	1165	1175	D6W5N0	MAGI2
516	RPQKTQsII	2136	2144	Q7Z333	SETX
517	RPRSGsTGSSL	2092	2102	Q5TH69	ARFGEF3
518	RPsNPQL	430	436	Q8IZJ1	UNC5B
519	RPSsGFYEL	156	164	Q9NYF0	DACT1
520	RPTsPIQIM	1002	1010	Q7Z7B0	FILIP1
524	SPDsSQSSL	105	113	F8W133	DDIT3
525	TDKYsKMM	220	227	Q6PI26	SHQ1
527	VPKSGRSSsL	1271	1280	Q9C0J8	WDR33
528	YPSsPRKAL	159	167	A6H8W6	SIPA1L1

Table 4

Exemplary Class I MHC Phosphopeptides on HCC that are Specific for HLA-B*2705

SEQ ID NO.	Sequence[#]	Start	Stop	UniProt Acc. No.	Gene Name
225	FRRsPTKSSL	624	633	Q96PK6	RBM14
226	FRRsPTKSSLD	624	634	Q96PK6	RBM14
227	FRRsPTKSSLDY	624	635	Q96PK6	RBM14
228	GRKsPPPSF	713	721	B4DLE8	CRYBG3
229	GRLsPAYSL	536	544	Q86UU1	PHLDB1
230	GRLsPVPVPR	132	141	Q9UKM9	RALY
231	GRQsPSFKL	738	746	Q6IN85	PPP4R3A
232	GRsSPPPGY	173	181	Q99759	MAP3K3
233	KRAsYILRL	2084	2092	Q96Q15	SMG1

234	KRFsFKKSF	156	164	P29966	MARCKS
235	KRFsFKKsF	156	164	P29966	MARCKS
236	KRFsGTVRL	47	55	P62906	RPL10A
237	KRKsFTSLY	955	963	Q5SW79	CEP170
238	KRLEKsPSF	656	664	Q92625	ANKS1A
239	KRLsPAPQL	51	59	Q9UH99	SUN2
240	KRmsPKPEL	17	25	P41208	CETN2
241	KRWQsPVTK	593	601	A9Z1X7	SRRM1
242	KRYsGNmEY	275	283	O95835	LATS1
243	KRYsRALYL	353	361	Q9UJX3	ANAPC7
244	QRLsPLSAAY	110	119	Q14774	HLX
245	RRAsIITKY	906	914	Q15849	SLC14A2
246	RRAsLSEIGF	177	186	Q00537	CDK17
247	RRDsIVAEL	96	104	O14579	COPE
248	RRDsLQKPGL	377	386	Q9NRM7	LATS2
249	RRFsGTAVY	652	660	Q6AHZ1	ZNF518A
250	RRFsIATLR	128	136	Q16696	CYP2A13
251	RRFsLTTLR	124	132	P10632	CYP2C8
252	RRFsPPRRm	248	256	Q15287	RNPS1
253	RRFsRSDEL	347	355	P18146	EGR1
254	RRFsRsPIR	2026	2034	P18583	SON
255	RRFSRsPIR	2026	2034	P18583	SON
256	RRFsRsPIRR	2026	2035	P18583	SON
257	RRGsFEVTL	75	83	Q8IZQ5	SELENOH
258	RRIDIsPSTF	677	686	Q9Y2W1	THRAP3
259	RRIsDPEVF	788	796	Q4L180	FILIP1L
260	RRIsDPQVF	788	796	Q4L180	FILIP1L
261	RRIsQIQQL	413	421	O60306	AQR
262	RRKsQVAEL	244	252	Q9BYG3	NIFK
263	RRLsADIRL	744	752	O60307	MAST3
264	RRLsELLRY	449	457	P08238	HSP90AB1
265	RRLsGGSHSY	332	341	Q13905	RAPGEF1

266	RRLsRKLSL	553	561	O75167	PHACTR2
267	RRMsFQKP	88	95	Q8N573	OXR1
268	RRmsLLSVV	314	322	Q9ULI2	RIMKLB
269	RRNsAPVSV	1175	1183	Q2M1Z3	ARHGAP31
270	RRPsIAPVL	687	695	Q5JUK3	KCNT1
271	RRPsLLSEF	67	75	O75376	NCOR1
272	RRPsLVHGY	31	39	P14324	FDPS
273	RRPsYTLGM	1629	1637	O43166	SIPA1L1
274	RRRsLERLL	1399	1407	Q96QZ7	MAGI1
275	RRSFsLE	1598	1604	Q12802	AKAP13
276	RRSsFLQ	585	591	Q15436	SEC23A
277	RRSsFLQVF	585	593	Q15436	SEC23A
278	RRSsIQSTF	231	239	Q92542	NCSTN
279	RRSsQSWSL	29	37	Q9Y4E1	WASHC2C
280	RRVVQRSsL	1138	1146	Q04637	EIF4G1
281	RRYsKFFDL	43	51	A1X283	SH3PXD2B
282	RRYsPPIQR	594	602	Q8IYB3	SRRM1
283	RSRsPLEL	23	30	Q92466	DDB2
284	SPRRsRSISL	159	168	Q16629	SRSF7
285	SRFNRRVsV	92	100	P13861	PRKAR2A

Table 5

Exemplary Class I MHC Phosphopeptides on HCC that are Specific for HLA-A*01

SEQ ID NO.	Sequence[#]	Start	Stop	UniProt Acc. No.	Gene Name
286	AEQGsPRVSY	2121	2130	Q01082	SPTBN1
287	GsPHYFSPFRPY	210	221	Q13242	SRSF9
288	ISSsMHSLY	222	230	P50616	TOB1
289	ITQGtPLKY	1459	1467	Q9Y618	NCOR2
290	LLDPSRSYsY	643	652	Q9H706	GAREM1
291	SLDsPSYVLY	57	66	P49354	FNTA
292	SLYDRPAsY	760	768	P16234	PDGFRA

293	SYPsPVATSY	441	450	P18146	EGR1
294	TMAsPGKDNY	3	12	O60684	KPNA6
295	YFsPFRPY	214	221	Q13242	SRSF9
296	YPLsPTKISQY	1197	1207	Q86Z02	HIPK1
297	YQRPFsPSAY	4	13	O94875	SORBS2

Table 6

Exemplary Class I MHC Phosphopeptides on HCC that are Specific for HLA-A*03

SEQ ID NO.	Sequence#	Start	Stop	UniProt Acc. No.	Gene Name
298	ATYtPQAPK	251	259	Q53GL0	PLEKHO1
299	FLIIRtVLQL	218	227	Q9H255	OR51E2
300	FRYsGKTEY	345	353	Q9HCM4	EPB41L5
301	GIMsPLAKK	253	261	Q03989	ARID5A
302	IISsPLTGK	461	469	Q9P275	USP36
303	ILKPRRsL	56	63	O15205	UBD
304	IYQyIQSRF	270	278	Q9Y463	DYRK1B
305	KLPDsPALA	571	579	Q13586	STIM1
306	KLPDsPALAK	571	580	Q13586	STIM1
307	KLPDsPALAKK	571	581	Q13586	STIM1
308	KLPsPAPARK	140	149	Q8IY33	MICALL2
309	KLRsPFLQK	280	288	Q9UJU6	DBNL
310	KMPTtPVKAK	47	56	Q8WUA7	TBC1D22A
311	KRAsVFVKL	153	161	P50502	ST13
312	KTPTsPLKMK	112	121	O60264	SMARCA5
313	KVQsLRRAL	185	193	Q969G5	PRKCDBP
314	MTRsPPRVSK	249	258	Q9BRL6	SRSF8
315	RAKsPISLK	509	517	Q9BXL7	CARD11
316	RILsGVVTK	71	79	P62280	RPS11
317	RIYQyIQ	269	275	Q9Y463	DYRK1B
318	RIYQyIQSR	269	277	Q9Y463	DYRK1B
319	RIYQyIQSRF	269	278	Q9Y463	DYRK1B

320	RLFVGsIPK	247	255	O43390	HNRNPR
321	RLLDRSPsRSAK	301	312	O76039	CDKL5
322	RLSsPISKR	327	335	Q99728	BARD1
323	RLSsPVLHR	139	147	Q16643	DBN1
324	RSLsVEIVY	863	871	Q9NS56	TOPORS
325	RSYsRSFSR	713	721	Q7Z6E9	RBBP6
326	RSYsYPRQK	648	656	Q9H706	GAREM1
327	RTAsFAVRK	240	248	Q9Y512	SAMM50
328	RTAsPPPPPK	586	595	M0R088	SRRM1
329	RTRsLSSLREK	1975	1985	O94915	FRYL
330	RVAsPTSGVK	1097	1106	Q9Y4H2	IRS2
331	RVKtPTSQSYR	885	895	Q9Y2X9	ZNF281
332	RVLsPLIIK	400	408	Q8NCN4	RNF169
333	RVRQsPLATR	40	49	O75381	PEX14
334	RVYsPYNHR	582	590	Q9NS56	TOPORS
335	SVKsPVTVK	329	337	Q9HCS4	TCF7L1
336	SVRRsVLMK	223	231	Q9H2J4	PDCL3
337	yIQSRF	273	278	Q9Y463	DYRK1B
511	KVLSPtAAK	310	318	Q96QC0	PPP1R10
522	RVRRsSFLNAK	61	71	H0Y8T6	RAPGEF2
523	RVWEDRPSsA	107	116	H7BZU2	NCOR2
526	VLDsPASKK	175	183	Q8N5I9	C12orf45

Table 7

Exemplary Class I MHC Phosphopeptides on HCC that are Specific for HLA-B*44

SEQ ID NO.	Sequence#	Start	Stop	UniProt Acc. No.	Gene Name
338	AENARSAsF	203	211	Q9UQC2	GAB2
339	AENsPTRQQF	93	102	Q86XP3	DDX42
340	AENsSSREL	567	575	P29590	PML
341	AtAGPRLGW	621	629	Q86W92	PPFIBP1

342	EELsPTAKF	117	125	Q99612	KLF6
343	FKtQPVTF	365	373	Q7Z7L8	C11orf96
344	GEAsPSHII	557	565	Q9ULL5	PRR12
345	GEIsPQREV	1023	1031	Q8WWI1	LMO7
346	GETsPRTKI	458	466	Q5VU08	ADD3
347	HEKKAYsF	215	222	Q15418	RPS6KA1
348	KEKsPFRET	1300	1308	Q9Y2F5	ICE1
349	KELARQIsF	177	185	Q9Y385	UBE2J1
350	KEmsPTRQL	36	44	Q4G0N7	FAM229B
351	KESsPLSSRKI	291	301	Q14693	LPIN1
352	REAPsPLmI	1060	1068	O60885	BRD4
353	REAsPAPLA	1199	1207	Q9P1Y6	PHRF1
354	REAsPRLRV	80	88	O00220	TNFRSF10A
355	REAsPSRLSV	504	513	O75122	CLASP2
356	REIMGtPEYL	193	202	O94768	STK17B
357	REKsPGRmL	1978	1986	O14578	CIT
358	RELARKGsL	57	65	Q8IW50	FAM219A
359	RELsPLISL	196	204	P51825	AFF1
360	REPsPLPEL	654	662	Q13207	TBX2
361	RERsPSPSF	326	334	P49585	PCYT1A
362	RESsPTRRL	158	166	Q9C0H9	SRCIN1
363	REVsPAPAV	1361	1369	O60292	SIPA1L3
364	REYGsTSSI	204	212	O43166	SIPA1L1
365	RFKtQPVTF	364	373	Q7Z7L8	C11orf96
366	RQKsPLFQF	240	248	Q8WY36	BBX
367	SEFKAMDsI	898	906	P35221	CTNNA1
368	SELsPGRSV	103	111	Q8NFF5	FLAD1
369	TEAsPESML	577	585	Q9H0E9	BRD8
370	YEGsPIKV	67	74	P06748	NPM1

Table 8

Exemplary Class I MHC Phosphopeptides on HCC that are Specific for HLA-C*06

SEQ ID NO.	Sequence#	Start	Stop	UniProt Acc. No.	Gene Name
371	FRFsGRTEY	309	317	Q9NX84	EPB41L4B
372	KRAsFAKSV	328	336	Q96J92	WNK4
373	LSSsVIREL	201	209	Q8NEJ9	NGDN
374	RKPsIVTKY	81	89	P46100	ATRX
375	RRHsASNLHAL	54	64	P47974	ZFP36L2
376	RRLsFLVSY	67	75	P47897	QARS
377	RRLsYVLFI	107	115	Q9HCL2	GPAM
378	RRPsYRKIL	133	141	Q03060	CREM
379	RSAsFSRKV	316	324	O75161	NPHP4
380	SRSSSVLsL	636	644	A1L390	PLEKHG3
381	TRKtPESFL	467	475	Q9Y6I3	EPN1
382	YRYsPQSFL	218	226	Q9HCM1	KIAA1551

Table 9

Exemplary Class I MHC Phosphopeptides on HCC that are Specific for HLA-C*05

SEQ ID NO.	Sequence#	Start	Stop	UniProt Acc. No.	Gene Name
383	KVDsPVIF	1114	1121	Q7Z401	DENND4A
384	RADsPVHM	444	451	O95402	MED26
385	RSDsYVEL	10	17	Q12888	TP53BP1
386	RSEsPPAEL	309	317	Q14669	TRIP12
387	RVDsPSHGL	685	693	Q9UER7	DAXX
388	SIDsPQKL	724	731	Q12888	TP53BP1
509	AAEsPSFL	97	104	Q53TG4	NCK2

Table 10

Exemplary Class I MHC Phosphopeptides on HCC that are Specific for HLA-A*24

SEQ ID NO.	Sequence#	Start	Stop	UniProt Acc. No.	Gene Name
389	RYQtQPVTL	849	857	O95425	SVIL
390	VYTyIQSRF	261	269	Q9NR20	DYRK4

Table 11

Exemplary Class I MHC Phosphopeptide on HCC that is Specific for HLA-A*31

SEQ ID NO.	Sequence#	Start	Stop	UniProt Acc. No.	Gene Name
391	RTSsFTFQN	440	448	P27540	ARNT

Table 12

Exemplary Class I MHC Phosphopeptide on HCC that is Specific for HLA-B*15

SEQ ID NO.	Sequence#	Start	Stop	UniProt Acc. No.	Gene Name
392	RAHsEPLAL	356	364	Q66K64	DCAF15

Table 13

Exemplary Class I MHC Phosphopeptides on HCC that are Specific for Untyped Class I HLA

SEQ ID NO.	Sequence#	Start	Stop	UniProt Acc. No.	Gene Name
393	ADLsPEREV	121	129	Q8TAI7	RHEBL1
394	AGDsPGSQF	284	292	Q12778	FOXO1
395	AKLsETIS	272	279	Q9ULJ1	ODF2L
396	AsLGFVF	115	121	Q8NCK7	SLC16A11
397	DAKKsPLAL	83	91	Q9H7S9	ZNF703
398	DLKSSKAsL	5742	5750	Q09666	AHNAK
399	FTKsPYQEF	261	269	P15880	RPS2
400	GQLsPGVQF	69	77	Q07002	CDK18

401	GsPHYFSPF	210	218	Q13242	SRSF9
402	HTAsPTGMMK	34	43	O94855	SEC24D
403	HVYtPSTTK	113	121	Q9H9E1	ANKRA2
404	IQFsPPFPGA	1353	1362	Q9Y2G9	SBNO2
405	KASPKRLsL	632	640	Q765P7	MTSS1L
406	KAVsLFLCY	4	12	P09912	IFI6
406	KAVsLFLcY	4	12	P09912	IFI6
407	KIFsGVFVK	114	122	Q6DKI1	RPL7L1
408	KIKsFEVVF	6	14	Q9H3M7	TXNIP
409	KLKDRLPsI	56	64	Q53QV2	LBH
410	KLsGDQPAAR	1348	1357	Q13428	TCOF1
411	KLSGLsF	99	105	P49006	MARCKSL1
412	KTMsPSQMIM	846	855	Q9ULJ6	ZMIZ1
413	KVKsSPLIEKL	79	89	Q6JBY9	RCSD1
414	NMDsPGPML	107	115	P32519	ELF1
415	PmVTLsLNL	160	168	Q8NDX9	LY6G5B
416	PYDPALGsPSR	58	68	Q6BEB4	SP5
417	RAFsVKFEV	113	121	P00973	OAS1
418	RGDGYGtF	587	594	Q9NQ94	A1CF
419	RIGsPLSPK	337	345	Q659C4	LARP1B
420	RKLRsLEQL	650	658	Q6NSJ5	LRRC8E
421	RKSsIIIRM	80	88	Q02325	PLGLB1
422	RLLDPsSPLAL	829	839	Q9UPX8	SHANK2
422	RLLDPSsPLAL	829	839	Q9UPX8	SHANK2
423	RLSsLRASTSK	233	243	P62753	RPS6
424	RMFsPMEEK	691	699	Q9UHB7	AFF4
425	RMYsPIPPSL	475	484	Q86TG7	PEG10
426	RNLsSPFIF	643	651	P52569	SLC7A2
427	RSRsPRPAL	?	?	Unknown	Unknown
428	RTHsLLLLL	5	13	P34096	RNASE4
429	RTNsPGFQK	515	523	Q5T8P6	RBM26
430	RTPsDVKEL	14	22	P39687	ANP32A

431	RTSsFALNL	3775	3783	P04114	APOB
432	RTSsPLFNK	125	133	Q9BY89	KIAA1671
433	RTYsHGTYR	451	459	Q6PCB5	RSBN1L
434	RYPsNLQLF	464	472	Q99973	TEP1
435	sDDEKMPDLE	151	160	Q15185	PTGES3
436	SDmPRAHsF	218	226	P78314	SH3BP2
437	SFDsGSVRL	413	421	O14896	IRF6
438	SsPIMRKKVSL	1171	1181	O43314	PPIP5K2
439	sYIEHIFEI	61	69	Q15121	PEA15
440	sYQKVIELF	289	297	Q96KB5	PBK
441	TLLAsPMLK	1248	1256	P17948	FLT1
442	TLMERTVsL	254	262	Q8IWE2	FAM114A1
443	VLFPEsPARA	4817	4826	O14686	KMT2D
444	VLIENVAsL	31	39	P18283	GPX2
445	VLSDVIPsI	151	159	Q6PEV8	FAM199X
446	VLVVDTPsI	78	86	Q96F15	GIMAP5
447	VVDsPGQEVL	22	31	Q8WUA4	GTF3C2
448	YARsVHEEF	354	362	Q9BRK5	SDF4
529	SARRtPVSY	1480	1488	O75376	NCOR1

Table 14

Exemplary Class I MHC Phosphopeptides on Esophageal Cancer

SEQ ID NO.	Sequence#	Start	Stop	UniProt Acc. No.	Gene Name
HLA A*0201					
502	SIPtVSGQI	15	23	Q8TF68	ZNF384
HLA A*0101					
503	YPLsPAKVNQY	1185	1195	Q9H2X6	HIPK2
504	YPLsPTKISEY	1197	1207	Q86Z02	HIPK1
HLA-B*0702					
505	VPLIRKKsL	20	28	B7ZW66	PGA5

Other HLA Alleles					
506	LKLsYLTWV	561	569	O43246	SLC7A4
507	KRYsEPVSL	647	655	Q9C0D6	FHDC1
508	KSGELLAtW	168	176	Q9H0K1	SIK2

Exemplary MHC class I phosphopeptides of the presently disclosed subject matter that are associated in some embodiments with esophageal cancer are set forth in Table 14 and as SEQ ID NOs: 502-508, for example.

In some embodiments, the phosphopeptides of the presently disclosed subject matter comprise the amino acid sequences of at least one of the MHC class I binding peptides set forth in SEQ ID NOs: 1-448 and 502-529. Moreover, in some embodiments about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more of the serine, homoserine, threonine, or tyrosine residues within the recited sequence is phosphorylated. The phosphorylation can in some embodiments be with a natural phosphorylation (-CH_2-O-PO_3H) or with an enzyme non-degradable, modified phosphorylation, such as (-CH_2-CF_2-PO_3H or –CH_2- CH_2-PO_3H). Some phosphopeptides can contain more than one of the amino acid sequences set forth in SEQ ID NOs: 1-448 and 502-529, for example, if they are overlapping, adjacent, or nearby within the native protein from which they are derived.

In some embodiments, the target peptides comprise a phosphopeptide mimetic. In some embodiments, the phosphopeptide mimetic replaces a phosphoserine, phosphothreonine, or phosphotyrosine residue indicated in Tables 2-14. The chemical structure of a phosphopeptide mimetic appropriate for use in the presently disclosed subject matter can in some embodiments closely approximate the natural phosphorylated residue which is mimicked, and also can in some embodiments be chemically stable (*e.g.*, resistant to dephosphorylation by phosphatase enzymes). This can be achieved with a synthetic molecule in which the phosphorous atom is linked to the amino acid residue, not through oxygen, but through carbon. In some embodiments, a CF_2 group links the amino acid to the phosphorous atom. Mimetics of several amino acids which are phosphorylated in nature can be generated by this approach. Mimetics of phosphoserine, phosphothreonine, and phosphotyrosine can be generated by placing a CF_2 linkage from the appropriate carbon to the phosphate moiety. The mimetic molecule L-2-amino-4 (diethylphosphono)-4,4-difluorobutanoic acid (F2Pab) can in some embodiments substitute for phosphoserine (Otaka *et al.*, 1995). L-2-amino-4-phosphono-4,4difluoro-3-

methylbutanoic acid (F2Pmb) can in some embodiments substitute for phosphothreonine. L-2-amino-4-phosphono (difluoromethyl) phenylalanine (F2Pmp) can in some embodiments substitute for phosphotyrosine (Smyth *et al.*, 1992; Akamatsu *et al.*, 1997). Alternatively, the oxygen bridge of the natural amino acid can in some embodiments be replaced with a methylene group. In some embodiments, serine and threonine residues are substituted with homo-serine and homo-threonine residues, respectively. A phosphomimetic can in some embodiments also include vanadate, pyrophosphate or fluorophosphates.

IV. Immunosuitablity

In some embodiments, the target peptides of the presently disclosed subject matter are combined into compositions which can be used in vaccine compositions for eliciting anti-tumor immune responses or in adoptive T-cell therapy of HCC patients and/or esophageal cancer patients. Tables 2-14 provide target peptides presented on the surface of cancer cells.

The presently disclosed subject matter provides in some embodiments target peptides which are immunologically suitable for each of the foregoing HLA alleles and, in particular, HLA-A*0201, HLA-B*0702, HLA-B*2705, HLA-A*01, HLA-A*03, HLA-B*44, HLA-C*06, HLA-C*05, HLA-A*24, HLA-A*31, and HLA-B*15. "Immunologically suitable" means that a target peptide will bind at least one allele of an MHC class I molecule in a given patient. Compositions of the presently disclosed subject matter are in some embodiments immunologically suitable for a patient when at least one target peptide of the composition will bind at least one allele of an MHC class I molecule in a given patient. Compositions of multiple target peptides presented by each of the most prevalent alleles used in a cocktail, ensures coverage of the human population and to minimize the possibility that the tumor will be able to escape immune surveillance by down-regulating expression of any one class I target peptide.

The compositions of the presently disclosed subject matter can in some embodiments have at least one target peptide specific for HLA-A*0201, HLA-B*0702, HLA-B*2705, HLA-A*01, HLA-A*03, HLA-B*44, HLA-C*06, HLA-C*05, HLA-A*24, HLA-A*31, and HLA-B*15. The compositions can in some embodiments have at least one phosphopeptide specific for an HLA allele selected from the group consisting of HLA-A*0201, HLA-B*0702, HLA-B*2705, HLA-A*01, HLA-A*03, HLA-B*44, HLA-C*06, HLA-C*05, HLA-A*24, HLA-A*31, and HLA-B*15. In some embodiments, the

compositions can further comprise additional phosphopeptides from other MHC class I alleles.

As such, the compositions of the presently disclosed subject matter containing various combinations of target peptides will in some embodiments be immunologically suitable for between or about 3-88%, 80-89%, 70-79%, 60-69%, 57-59%, 55-57%, 53-55% or 51-53% or 5-90%, 10-80%, 15-75%, 20-70%, 25-65%, 30-60%, 35-55%, or 40-50% of the population of a particular cancer, *e.g.*, HCC. In some embodiments, the compositions of the presently disclosed subject matter are able to act as vaccine compositions for eliciting anti-tumor immune responses or in adoptive T-cell therapy of HCC patients, wherein the compositions are immunologically suitable for about or at least 88, 87, 86, 85, 84, 83, 82, 81, 80, 79, 78, 77, 76,75, 74, 73, 72, 71, 70, 69, 68, 67, 66, 65, 64, 63, 62, 61, 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4 or 3 percent of cancer, *e.g.*, HCC, patients.

V. Compositions

"Target peptide compositions" as used herein refers to at least one target peptide formulated for example, as a vaccine; or as a preparation for pulsing cells in a manner such that the pulsed cells, *e.g.*, dendritic cells, will display the at least one target peptide in the composition on their surface, *e.g.*, to T-cells in the context of adoptive T-cell therapy.

The compositions of the presently disclosed subject matter can include in some embodiments about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 50-55, 55-65, 65-80, 80-120, 90-150, 100-175, or 175-250 different target peptides.

The compositions of the presently disclosed subject matter generally include MHC class I specific target peptide(s) but in some embodiments can also include one or more target peptides specific for MHC class II or other peptides associated with tumors, *e.g.*, tumor-associated antigen ("TAA").

Compositions comprising the presently disclosed target peptide are typically substantially free of other human proteins or peptides. They can be made synthetically or by purification from a biological source. They can be made recombinantly. In some embodiments, they are at least 90%, 92%, 93%, 94%, at least 95%, or at least 99% pure. For administration to a human body, in some embodiments they do not contain other components that might be harmful to a human recipient. The compositions are typically

devoid of cells, both human and recombinant producing cells. However, as noted below, in some cases, it can be desirable to load dendritic cells with a target peptide and use those loaded dendritic cells as either an immunotherapy agent themselves, or as a reagent to stimulate a patient's T cells *ex vivo*. The stimulated T cells can be used as an immunotherapy agent. In some embodiments, it can be desirable to form a complex between a target peptide and an HLA molecule of the appropriate type. Such complexes can in some embodiments be formed *in vitro* or *in vivo*. Such complexes are typically tetrameric with respect to an HLA-target peptide complex. Under certain circumstances it can be desirable to add additional proteins or peptides, for example, to make a cocktail having the ability to stimulate an immune response in a number of different HLA type hosts. Alternatively, additional proteins or peptide can provide an interacting function within a single host, such as an adjuvant function or a stabilizing function. As a non-limiting example, other tumor antigens can be used in admixture with the target peptides, such that multiple different immune responses are induced in a single patient.

Administration of target peptides to a mammalian recipient can in some embodiments be accomplished using long target peptides (*e.g.*, longer than 15 residues) or using target peptide loaded dendritic cells (*see* Melief, 2009). The immediate goal is to induce activation of $CD8^+$ T cells. Additional components which can be administered to the same patient, either at the same time or close in time (*e.g.*, within 21 days of each other) include TLR-ligand oligonucleotide CpG and related target peptides that have overlapping sequences of at least 6 amino acid residues. To ensure efficacy, mammalian recipients should express the appropriate human HLA molecules to bind to the target peptides. Transgenic mammals can be used as recipients, for example, if they express appropriate human HLA molecules. If a mammal's own immune system recognizes a similar target peptide then it can be used as model system directly, without introducing a transgene. Useful models and recipients can in some embodiments be at increased risk of developing metastatic cancer, such as HCC. Other useful models and recipients can be predisposed, *e.g.*, genetically or environmentally, to develop HCC or other cancer.

V.A. Selection of Target Peptides

Disclosed herein is the finding that immune responses can be generated against phosphorylated peptides tested in healthy and diseased individuals. The T-cells associated with these immune responses, when expanded *in vitro*, are able to recognize and kill malignant tissue (both established cells lines and primary tumor samples). Cold-target

inhibition studies reveal that these target peptide-specific T-cell lines kill primary tumor tissue in a target peptide-specific manner.

When selecting target peptides of the presently disclosed subject matter for inclusion in immunotherapy, *e.g.*, in adaptive cell therapy or in the context of a vaccine, one can preferably pick target peptides that in some embodiments: 1) are associated with a particular cancer/tumor cell type; 2) are associated with a gene/protein involved in cell proliferation; 3) are specific for an HLA allele carried the group of patients to be treated; and/or 4) are capable of inducing a target peptide-specific memory T cell response in the patients to be treated upon a first exposure to a composition including the selected target peptides.

V.B. Target Peptide Vaccines

The antigen target peptides can also in some embodiments be used to vaccinate an individual. The antigen target peptides can be injected alone or in some embodiments can be administered in combination with an adjuvant and a pharmaceutically acceptable carrier. Vaccines are envisioned to prevent or treat certain diseases in general and cancers in particular.

The target peptides compositions of the presently disclosed subject matter can in some embodiments be used as a vaccine for cancer, and more specifically for hepatocellular carcinoma (HCC), esophageal cancer, melanoma, leukemia, ovarian, breast, colorectal, or lung squamous cancer, sarcoma, renal cell carcinoma, pancreatic carcinomas, squamous tumors of the head and neck, brain cancer, liver cancer, prostate cancer, and cervical cancer. The compositions can in some embodiments include target peptides. The vaccine compositions can in some embodiments include only the target peptides, or peptides disclosed herein, or they can include other cancer antigens that have been identified.

The vaccine compositions can in some embodiments be used prophylactically for the purposes of preventing, reducing the risk of, and/or delaying initiation of a cancer in an individual that does not currently have cancer. Alternatively, they can be used to treat an individual that already has cancer, so that recurrence or metastasis is delayed and/or prevented. Prevention relates to a process of prophylaxis in which the individual is immunized prior to the induction or onset of cancer. For example, individuals with a history of poor life style choices and at risk for developing HCC can in some embodiments be immunized prior to the onset of the disease.

Alternatively or in addition, individuals that already have cancer can be immunized with the antigens of the presently disclosed subject matter so as to stimulate an immune response that would be reactive against the cancer. A clinically relevant immune response would be one in which the cancer partially or completely regresses and/or is eliminated from the patient, and it would also include those responses in which the progression of the cancer is blocked without being eliminated. Similarly, prevention need not be total, but can in some embodiments result in a reduced risk, delayed onset, and/or delayed progression or metastasis.

The target peptide vaccines of the presently disclosed subject matter can in some embodiments be given to patients before, after, or during any of the aforementioned stages of HCC and/or esophageal cancer. In some embodiments, they are given to patients with malignant HCC and/or malignant esophageal cancer (*e.g.*, squamous cell carcinoma and/or adenocarcinoma).

In some embodiments, the 5-year survival rate of patients treated with the vaccines of the presently disclosed subject matter is increased by a statistically significant amount, *e.g.*, by about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or more percent, relative to the average 5-year survival rates described above.

In some embodiments, the target peptide vaccine composition of the presently disclosed subject matter will increase survival rates in patients with metastatic HCC and/or malignant esophageal cancer by a statistically significant amount of time, *e.g.*, by about or at least, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 4.0, 4.25, 4.5, 4.75, 5.0, 5.25, 5.5, 5.75, 6.0, 6.25, 6.5, 6.75, 7.0, 7.25, 7.5, 7.75, 8.0, 8.25, 8.5, 8.75, 9.0, 9.25, 9.50, 9.75, 10.0, 10.25, 10.5, 10.75, 11.0, 11.25, 11.5, 11.75, or 12 months or more compared to what could have been expected without vaccine treatment at the time of filing of this disclosure.

In some embodiments, the survival rate, *e.g.*, the 1, 2, 3, 4, or 5-year survival rate, of patients treated with the vaccines of the presently disclosed subject matter is increased by a statistically significant amount, *e.g.*, by about, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58,

59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 percent, relative to the average 5-year survival rates described above.

The target peptide vaccines of the presently disclosed subject matter are in some embodiments envisioned to illicit a T cell associated immune response, *e.g.*, generating activated $CD8^+$ T cells specific for native target peptide/MHC class I expressing cells, specific for at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more of the target peptides in the vaccine in a patient for about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 07, 98, 99, or 100 days after providing the vaccine to the patient.

In some embodiments, the treatment response rates of patients treated with the target peptide vaccines of the presently disclosed subject matter are increased by a statistically significant amount, *e.g.*, by about, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 07, 98, 99, 100, 150, 200, 250, 300, 350, 400, 450, 500, or more percent, relative to treatment without the vaccine.

In some embodiments, overall median survival of patients treated with the target peptide vaccines of the presently disclosed subject matter is increased by a statistically significant amount, *e.g.*, by about, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 150, 200, 250, 300, 350, 400, 450, 500, or more percent, relative to treatment without the vaccine. In some embodiments, the overall median survival of HCC patients treated the target peptide vaccines is envisioned to be about or at least 10.0, 10.25, 10.5, 10.75, 11.0, 11.25, 11.5, 11.75, 12, 12.25, 12.5, 12.75, 13, 13.25, 13.5, 13.75, 14, 14.25, 14.5, 14.75, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, or more months.

In some embodiments, tumor size of patients treated with the target peptide vaccines of the presently disclosed subject matter is decreased by a statistically significant amount, *e.g.*, by about, or by at least, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 150, 200, 250, 300, 350, 400, 450, 500, or more percent, relative to treatment without the vaccine.

In some embodiments, the compositions of the presently disclosed subject matter provide an clinical tumor regression by a statistically significant amount, *e.g.*, in about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 percent of patients treated with a composition of the presently disclosed subject matter.

In some embodiments, the compositions of the presently disclosed subject matter provide a CTL response specific for the cancer being treated (such as but not limited to HCC and/or malignant esophageal cancer) by a statistically significant amount, *e.g.*, in about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 percent of patients treated with a composition of the presently disclosed subject matter.

In some embodiments, the compositions of the presently disclosed subject matter provide an increase in progression free survival in the cancer being treated (*e.g.*, HCC and/or malignant esophageal cancer), of about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, or more

percent compared to the progression free survival or patients not treated with the composition.

In some embodiments, progression free survival, CTL response rates, clinical tumor regression rates, tumor size, survival rates (including but not limited to overall survival rates), and/or response rates are determined, assessed, calculated, and/or estimated weekly, monthly, bi-monthly, quarterly, semi-annually, annually, and/or bi-annually over a period of about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more years or about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, or more weeks.

V.C. Compositions for Priming T cells

Adoptive cell transfer is the passive transfer of cells, in some embodiments immune-derived cells, into a recipient host with the goal of transferring the immunologic functionality and characteristics into the host. Clinically, this approach has been exploited to transfer either immune-promoting or tolergenic cells (often lymphocytes) to patients to enhance immunity against cancer. The adoptive transfer of autologous tumor infiltrating lymphocytes (TIL) or genetically re-directed peripheral blood mononuclear cells has been used to successfully treat patients with advanced solid tumors, including melanoma and ovarian carcinoma, HCC, and/or malignant esophageal cancer (*e.g.*, squamous cell carcinoma and/or adenocarcinoma), as well as patients with CD19-expressing hematologic malignancies. In some embodiments, adoptive cell transfer (ACT) therapies achieve T-cell stimulation *ex vivo* by activating and expanding autologous tumor-reactive T-cell populations to large numbers of cells that are then transferred back to the patient (*see e.g.*, Gattinoni *et al.*, 2006).

The target peptides of the presently disclosed subject matter can in some embodiments take the form of antigen peptides formulated in a composition added to autologous dendritic cells and used to stimulate a T helper cell or CTL response *in vitro*. The *in vitro* generated T helper cells or CTL can then be infused into a patient with cancer (Yee *et al.*, 2002), and specifically a patient with a form of cancer that expresses one or more of antigen target peptides.

Alternatively or in addition, the target peptides of the presently disclosed subject matter can be added to dendritic cells *in vitro*, with the loaded dendritic cells being subsequently transferred into an individual with cancer in order to stimulate an immune response. Alternatively or in addition, the loaded dendritic cells can be used to stimulate $CD8^+$ T cells *ex vivo* with subsequent reintroduction of the stimulated T cells to the patient. Although a particular target peptide can be identified on a particular cancer cell type, it can be found on other cancer cell types.

The presently disclosed subject matter envisions treating cancer by providing a patient with cells pulsed with a composition of target peptides. The use of dendritic cells ("DCs") pulsed with target peptide antigens allows for manipulation of the immunogen in two ways: varying the number of cells injected and varying the density of antigen presented on each cell. Exemplary methods for DC-based based treatments can be found for example in Mackensen *et al.*, 2000.

V.D. Additional Peptides Present in Target Peptide Compositions

The target peptide compositions (or target peptide composition kits) of the presently disclosed subject matter can in some embodiments also include at least one additional peptide derived from tumor-associated antigens. Examples of tumor-associated antigens include MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGE), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, β-HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein/cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, prostatic acid phosphatase, and the like. Particular examples of additional peptides derived from tumor-associated antigens that can be employed alone or in combination with the compositions of the presently disclosed subject matter those set forth in Table 15 below.

Table 15

Exemplary Peptides Derived from Tumor-associated Antigens

Polypeptide Name[a]	Amino Acid Sequence[b] (SEQ ID NO:)	GENBANK® Acc. No(s).[c]
CEA_{61-69}	HLFGYSWYK (SEQ ID NO: 452)	NP_001264092.1 XP_005278431.1
$CEA_{604-612}$	YLSGADLNL (SEQ ID NO: 453)	XP_005278431.1
$FBP/FOLR1_{191-199}$	EIWTHSYKV (SEQ ID NO: 454)	NP_000793.1
$gp100_{17–25}$	ALLAVGATK (SEQ ID NO: 455)	NP_001186982.1
$gp100_{44–59}$	WNRQLYPEWTEAQRLD (SEQ ID NO: 456)	NP_008859.1
$gp100_{87-95}$	ALNFPGSQK (SEQ ID NO: 457)	NP_008859.1
$gp100_{89-95}$	SQNFPGSQK (SEQ ID NO: 458)	NP_008859.1
$gp100_{154–162}$	KTWGQYWQV (SEQ ID NO: 459)	NP_008859.1
$gp100_{209–217}$	ITDQVPFSV (SEQ ID NO: 460)	NP_008859.1
$gp100_{209–217}$	IMDQVPFSV (SEQ ID NO: 461)	NP_008859.1
$gp100_{280–288}$	YLEPGPVTA (SEQ ID NO: 462)	NP_008859.1
$gp100_{476–485}$	VLYRYGSFSV (SEQ ID NO: 463)	NP_008859.1
$gp100_{614-622}$	LIYRRRLMK (SEQ ID NO: 464)	NP_008859.1
$Her2/neu_{369-377}$	KIFGSLAFL (SEQ ID NO: 465)	NP_004439.2
$Her2/neu_{754-762}$	VLRENTSPK (SEQ ID NO: 466)	NP_004439.2
$MAGE-A1_{114-127}$ $MAGE-A2,3,6_{121–134}$	LLKYRAREPVTKAE (SEQ ID NO: 467)	NP_004979.3 NP_005352.1 NP_005353.1 NP_005354.1
$MAGE-A1_{96-104}$	SLFRAVITK (SEQ ID NO: 468)	NP_004979.3
$MAGE-A1_{161–169}$	EADPTGHSY (SEQ ID NO: 469)	NP_004979.3
$MAGE-A3_{168–176}$	EVDPIGHLY (SEQ ID NO: 470)	NP_005353.1
$MAGE-A3_{281–295}$	TSYVKVLHHMVKISG (SEQ ID NO: 471)	NP_005353.1
$MAGE-A10_{254–262}$	GLYDGMEHL (SEQ ID NO: 472)	NP_001011543.2
$MART-1/MelanA_{27–35}$	AAGIGILTV (SEQ ID NO: 473)	NP_005502.1

MART-1/MelanA$_{51–73}$	RNGYRALMDKSLHVGTQCALTRR (SEQ ID NO: 474)	NP_005502.1
MART-1/MelanA$_{97-116}$	VPNAPPAYEKLsAEQSPPPY (SEQ ID NO: 475)	NP_005502.1
MART-1/MelanA$_{98-109}$	PNAPPAYEKLsA (SEQ ID NO: 476)	NP_005502.1
MART-1/MelanA$_{99-110}$	NAPPAYEKLsAE (SEQ ID NO: 477)	NP_005502.1
MART-1/MelanA$_{100-108}$	APPAYEKLs (SEQ ID NO: 478)	NP_005502.1
MART-1/MelanA$_{100-111}$	APPAYEKLsAEQ (SEQ ID NO: 479)	NP_005502.1
MART-1/MelanA$_{100-114}$	APPAYEKLsAEQSPP (SEQ ID NO: 480)	NP_005502.1
MART-1/MelanA$_{100-115}$	APPAYEKLsAEQSPPP (SEQ ID NO: 481)	NP_005502.1
MART-1/MelanA$_{100-116}$	APPAYEKLsAEQSPPPY (SEQ ID NO: 482)	NP_005502.1
MART-1/MelanA$_{101-109}$	PPAYEKLsA (SEQ ID NO: 483)	NP_005502.1
MART-1/MelanA$_{101-112}$	PPAYEKLsAEQS (SEQ ID NO: 484)	NP_005502.1
MART-1/MelanA$_{102-110}$	PAYEKLsAE (SEQ ID NO: 485)	NP_005502.1
MART-1/MelanA$_{102-113}$	PAYEKLsAEQSP (SEQ ID NO: 486)	NP_005502.1
MART-1/MelanA$_{103-114}$	AYEKLsAEQSPP (SEQ ID NO: 487)	NP_005502.1
MART-1/MelanA$_{104-115}$	YEKLsAEQSPPP (SEQ ID NO: 488)	NP_005502.1
NY-ESO-1	AAQERRVPR (SEQ ID NO: 489)	AAD05203.1 CAA10193.1
NY-ESO-1	LLGPGRPYR (SEQ ID NO: 490)	NP_001913.2
NY-ESO-1$_{53-62}$	ASGPGGGAPR (SEQ ID NO: 491)	NP_001318.1
p2$_{830-844}$	AQYIKANSKFIGITEL (SEQ ID NO: 492)	NP_783831.1
TAG-1,2	RLSNRLLLR (SEQ ID NO: 493)	
Tyr$_{56–70}$	AQNILLSNAPLGPQFP (SEQ ID NO: 494)	NP_000363.1
Tyr$_{146–156}$	SSDYVIPIGTY (SEQ ID NO: 495)	NP_000363.1
Tyr$_{240–251}$	SDAEKSDICTDEY (SEQ ID NO: 496)	NP_000363.1

$Tyr_{243-251}$	KCDICTDEY (SEQ ID NO: 497)	NP_000363.1
$Tyr_{369-377}$	YMDGTMSQV (SEQ ID NO: 498)	NP_000363.1
$Tyr_{388-406}$	FLLHHAFVDSIFEQWLQRHRP (SEQ ID NO: 499)	NP_000363.1

[a] Numbers listed in subscript are the amino acids positions of the listed peptide sequence in the corresponding polypeptide including, but not limited to the amino acid sequences provided in the GENBANK® biosequence database.

[b] lower case amino acids in this column are optionally phosphorylated.

[c] GENBANK® biosequence database Accession Numbers listed here are intended to be exemplary only and should not be interpreted to limit the disclosed peptide sequences to only these polypeptides.

Such tumor specific peptides (including the MHC class I phosphopeptides disclosed in SEQ ID NOs: 1-448 and 502-529 and in Tables 2-14) can be added to the target peptide compositions in a manner, number, and/or in an amount as if they were an additional target peptide added to the target peptide compositions as described herein.

V.E. Combination Therapies

In some embodiments, the target peptide compositions (or target peptide composition kits) of the presently disclosed subject matter are administered as a vaccine or in the form of pulsed cells as first, second, third, or fourth line treatment for the cancer. In some embodiments, the compositions of the presently disclosed subject matter are administered to a patient in combination with one or more therapeutic agents, *e.g.*, anti-CA125 (or oregovomab Mab B43.13), anti-idiotype Ab (ACA-125), anti-HER-2 (trastuzumab, pertuzumab), anti-MUC-1 idiotypic Ab (HMFG1), HER-2/neu peptide, NY-ESO-1, anti-Programed Death-1 ("PD1") (or PD1-antagonists such as BMS-936558), anti-CTLA-4 (or CTLA-4 antagonists), vermurafenib, ipilimumab, dacarbazine, IL-2, IFN-α, IFN-γ, temozolomide, receptor tyrosine kinase inhibitors (*e.g.*, imatinib, gefitinib, erlotinib, sunitinib, tyrphostins, telatinib), sipileucel-T, tumor cells transfected with GM-CSF, a platinum-based agent, a taxane, an alkylating agent, an antimetabolite and/or a vinca alkaloid or combinations thereof. In an embodiment, the cancer is sensitive to or refractory, relapsed or resistant to one or more chemotherapeutic agents, *e.g.*, a platinum-based agent, a taxane, an alkylating agent, an anthracycline (*e.g.*, doxorubicin (*e.g.*, liposomal doxorubicin)), an antimetabolite and/or a vinca alkaloid. In some embodiments, the cancer is, *e.g.*, HCC, and the HCCis refractory, relapsed, or resistant to a platinum-

based agent (*e.g.*, carboplatin, cisplatin, oxaliplatin), a taxane (*e.g.*, paclitaxel, docetaxel, larotaxel, cabazitaxel) and/or an anthracycline (*e.g.*, doxorubicin (*e.g.*, liposomal doxorubicin)). In some embodiments, the cancer is, *e.g.*, HCC, and the HCC is refractory, relapsed, or resistant to an antimetabolite (*e.g.*, an antifolate (*e.g.*, pemetrexed, floxuridine, raltitrexed) and a pyrimidine analogue (*e.g.*, capecitabine, cytrarabine, gemcitabine, 5FU)) and/or a platinum-based agent (*e.g.*, carboplatin, cisplatin, oxaliplatin). In some embodiments, the cancer is, *e.g.*, lung cancer, and the cancer is refractory, relapsed or resistant to a taxane (*e.g.*, paclitaxel, docetaxel, larotaxel, cabazitaxel), a platinum-based agent (*e.g.*, carboplatin, cisplatin, oxaliplatin), a vinca alkaloid (*e.g.*, vinblastine, vincristine, vindesine, vinorelbine), a vascular endothelial growth factor (VEGF) pathway inhibitor, an epidermal growth factor (EGF) pathway inhibitor) and/or an antimetabolite (*e.g.*, an antifolate (*e.g.*, pemetrexed, floxuridine, raltitrexed) and a pyrimidine analogue (*e.g.*, capecitabine, cytrarabine, gemcitabine, 5FU)). In some embodiments, the cancer is, *e.g.*, breast cancer, and the cancer is refractory, relapsed or resistant to a taxane (*e.g.*, paclitaxel, docetaxel, larotaxel, cabazitaxel), a vascular endothelial growth factor (VEGF) pathway inhibitor, an anthracycline (*e.g.*, daunorubicin, doxorubicin (*e.g.*, liposomal doxorubicin), epirubicin, valrubicin, idarubicin), a platinum-based agent (*e.g.*, carboplatin, cisplatin, oxaliplatin), and/or an antimetabolite (*e.g.*, an antifolate (*e.g.*, pemetrexed, floxuridine, raltitrexed) and a pyrimidine analogue (*e.g.*, capecitabine, cytrarabine, gemcitabine, 5FU)). In some embodiments, the cancer is, *e.g.*, gastric cancer, and the cancer is refractory, relapsed or resistant to an antimetabolite (*e.g.*, an antifolate (*e.g.*, pemetrexed, floxuridine, raltitrexed) and a pyrimidine analogue (*e.g.*, capecitabine, cytrarabine, gemcitabine, 5FU)) and/or a platinum-based agent (*e.g.*, carboplatin, cisplatin, oxaliplatin).

In some embodiments, the target peptide compositions (or target peptide composition kits) of the presently disclosed subject matter are associated with agents that inhibit T cell apoptosis or anergy thus potentiating a T cell response ("T cell potentiator"). Such agents include B7RP1 agonists, B7-H3 antagonists, B7-H4 antagonists, HVEM antagonists, HVEM antagonists, GAL9 antagonists or alternatively CD27 agonists, OX40 agonists, CD137 agonists, BTLA agonists, ICOS agonists CD28 agonists, or soluble versions of PDL1, PDL2, CD80, CD96, B7RP1, CD137L, OX40 or CD70. *See* Pardoll, National Reviews of Cancer, Focus on Tumor Immunology & Immunotherapy, 254, April 2012, Volume 12.

In some embodiments, the T cell potentiator is a PD1 antagonist. Programmed death 1 (PD-1) is a key immune checkpoint receptor expressed by activated T cells, and it mediates immunosuppression. PD-1 functions primarily in peripheral tissues, where T cells can encounter the immunosuppressive PD-1 ligands PD-L1 (B7-H1) and PD-L2 (B7-DC), which are expressed by tumor cells, stromal cells, or both. In some embodiments, the anti–PD-1 monoclonal antibody BMS-936558 (also known as MDX-1106 and ONO-4538) is used. In some embodiments, the T cell potentiator, *e.g.*, PD1 antagonist, is administered as an intravenous infusion at least or about every 1, 1.5, 2, 2.5, 3, 3.5, or 4 weeks of each 4, 5, 6, 7, 8, 9, or 10-week treatment cycle of about for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more cycles. Exemplary, non-limiting doses of the PD1 antagonists are envisioned to be exactly, about, or at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13,14, 15, 16, 17, 18, 19, 20, or more mg/kg (*see* Brahmer *et al.*, 2012).

The exemplary therapeutic agents disclosed herein above are envisioned to be administered at a concentration of, *e.g.*, about 1 to 100 mg/m^2, about 10 to 80 mg/m^2, about 40 to 60 mg/m^2, *e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or more mg/mm^2. Alternatively, the exemplary therapeutic agents disclosed herein above are envisioned to be administered at a concentration of, *e.g.*, about or at least 0.001 to 100 mg/kg or 0.1 to 1 mg/kg. In some embodiments, the exemplary therapeutic agents disclosed herein above are envisioned to be administered at a concentration of, *e.g.*, about or at least from 0.01 to 10 mg/kg.

The target peptide compositions (or target peptide composition kits) of the presently disclosed subject matter can in some embodiments also be provided with administration of cytokines such as lymphokines, monokines, growth factors and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; prostaglandin, fibroblast growth factor; prolactin; placental lactogen, OB protein; tumor necrosis factor-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide;

inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha -beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, LIF, G-CSF, GM-CSF, M-CSF, EPO, kit-ligand or FLT-3, angiostatin, thrombospondin, endostatin, tumor necrosis factor and LT. As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The target peptide compositions of the presently disclosed subject matter can in some embodiments be provided with administration of cytokines around the time, (*e.g.*, about or at least 1, 2, 3, or 4 weeks or days before or after) of the initial dose of a target peptide composition.

Exemplary, non-limiting doses of a cytokine would be about or at least 1-100, 10-80, 20-70, 30-60, 40-50, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 Mu/m^2/day over about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 days. The cytokine can in some embodiments be delivered at least or about once every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours. Cytokine treatment can in some embodiments be provided in at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 cycles of at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks, wherein each cycle has at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 cytokine doses. Cytokine treatment can be on the same schedule as administration of the target peptide compositions or on a different (but in some embodiments overlapping) schedule.

In some embodiments, the cytokine is IL-2 and is dosed in an amount of about or at least 100,000 to 1,000,000; 200,000-900,000; 300,000-800,000; 450,000-750,000; 600,000-800,000; or 700,000-800,000; or 720,000 units (IU)/kg administered, *e.g.*, as a

bolus, every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 hours for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days, in a cycle, for example.

VI Types of Proliferative Disease

The compositions of the presently disclosed subject matter are envisioned to useful in the treatment of benign and malignant proliferative diseases. Excessive proliferation of cells and turnover of cellular matrix can contribute significantly to the pathogenesis of several diseases, including but not limited to cancer, atherosclerosis, rheumatoid arthritis, psoriasis, idiopathic pulmonary fibrosis, scleroderma and cirrhosis of the liver, ductal hyperplasia, lobular hyperplasia, papillomas, and others.

In some embodiments, the proliferative disease is cancer, which in some embodiments is selected from the group consisting of HCC, esophageal cancer, breast cancer, colorectal cancer, squamous carcinoma of the lung, sarcoma, renal cell carcinoma, pancreatic carcinomas, squamous tumors of the head and neck, leukemia, brain cancer, liver cancer, prostate cancer, ovarian cancer, and cervical cancer. In some embodiments, the compositions of the presently disclosed subject matter are used to treat HCC, esophageal cancer, colorectal cancer, acute myelogenous leukemia (AML), acute lyphocytic leukemia (ALL), chronic lymphocytic lymphoma (CLL), chronic myelogenous leukemia (CML), breast cancer, renal cancer, pancreatic cancer, and/or ovarian cancer.

In some embodiments, the cancer is a cancer of the bladder (including accelerated and metastatic bladder cancer), breast (*e.g.*, estrogen receptor positive breast cancer, estrogen receptor negative breast cancer, HER-2 positive breast cancer, HER-2 negative breast cancer, triple negative breast cancer, inflammatory breast cancer), colon (including colorectal cancer), kidney (*e.g.*, renal cell carcinoma), liver, lung (including small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma)), genitourinary tract, *e.g.*, ovary (including fallopian, endometrial and peritoneal cancers), cervix, prostate and testes, lymphatic system, rectum, larynx, pancreas (including exocrine pancreatic carcinoma), stomach (*e.g.*, gastroesophageal, upper gastric or lower gastric cancer), gastrointestinal cancer (*e.g.*, anal cancer), gall bladder, thyroid, lymphoma (*e.g.*, Burkitt's, Hodgkin's, or non-Hodgkin's lymphoma), leukemia (*e.g.*, acute myeloid leukemia), Ewing's sarcoma, nasoesophageal cancer, nasopharyngeal cancer, neural and glial cell cancers (*e.g.*, glioblastoma multiforme), and head and neck. Exemplary cancers include but are not limited to HCC, esophageal cancer (including Barrett's esophagus (BE), high-grade dysplasia (HGD), and invasive cancer including but not limited to squamous cell

carcinoma and adenocarcinoma), melanoma, breast cancer (*e.g.*, metastatic or locally advanced breast cancer), prostate cancer (*e.g.*, hormone refractory prostate cancer), renal cell carcinoma, lung cancer (*e.g.*, small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma)), pancreatic cancer, gastric cancer (*e.g.*, gastroesophageal, upper gastric or lower gastric cancer), colorectal cancer, squamous cell cancer of the head and neck, ovarian cancer (*e.g.*, advanced ovarian cancer, platinum-based agent resistant or relapsed ovarian cancer), lymphoma (*e.g.*, Burkitt's, Hodgkin's, or non-Hodgkin's lymphoma), leukemia (*e.g.*, acute myeloid leukemia), and gastrointestinal cancer.

VII. Administration of Vaccine Compositions

VII.A. Routes of Administration

The target peptide compositions of the presently disclosed subject matter can in some embodiments be administered parenterally, systemically, and/or topically. By way of example and not limitation, composition injection can be performed by intravenous (i.v). injection, sub-cutaneous (s.c). injection, intradermal (i.d). injection, intraperitoneal (i.p). injection, and/or intramuscular (i.m). injection. One or more such routes can be employed. Parenteral administration can be, for example, by bolus injection or by gradual perfusion over time. Alternatively or concurrently, administration can be by the oral route.

In some embodiments, intradermal (i.d). injection is employed. The target peptide compositions of the presently disclosed subject matter are suitable for administration of the peptides by any acceptable route such as oral (enteral), nasal, ophthal, or transdermal. In some embodiments, the administration is subcutaneous and can be administered by an infusion pump.

VII.B. Formulation

Pharmaceutical carriers, diluents, and excipients are generally added to the target peptide compositions or (target peptide compositions kits) that are compatible with the active ingredients and acceptable for pharmaceutical use. Examples of such carriers include, but are not limited to, water, saline solutions, dextrose, and/or glycerol. Combinations of carriers can also be used. The vaccine compositions can further incorporate additional substances to stabilize pH and/or to function as adjuvants, wetting agents, and/or emulsifying agents, which can serve to improve the effectiveness of the vaccine.

The target peptide compositions can include one or more adjuvants such but not limited to montanide ISA-51 (Seppic, Inc., Fairfield, New Jersey, United States of

America); QS-21 STIMULON® brand adjuvant (Agenus Inc., Lexington, Massachusetts, United States of America); ARLACEL® A brand mannide monooleate; oeleic acid; tetanus helper peptides (*e.g.*, QYIKANSKFIGITEL (SEQ ID NO: 449) or AQYIKANSKFIGITEL (SEQ ID NO: 450); GM-CSF; cyclophosamide; bacillus Calmette-Guerin (BCG); corynbacterium parvum; levamisole, azimezone; isoprinisone; dinitrochlorobenezene (DNCB); keyhole limpet hemocyanins (KLH) including Freunds adjuvant (complete and incomplete); mineral gels; aluminum hydroxide (Alum); lysolecithin; pluronic polyols; polyanions; peptides; oil emulsions; nucleic acids (*e.g.*, dsRNA) dinitrophenol; diphtheria toxin (DT); toll-like receptor (TLR, *e.g.*, TLR3, TLR4, TLR7, TLR8 or TLR9) agonists (e.g, endotoxins such as lipopolysaccharide (LPS); monophosphoryl lipid A (MPL); polyinosinic-polycytidylic acid (poly-ICLC/HILTONOL®; Oncovir, Inc., Washington, DC, United States of America); IMO-2055; glucopyranosyl lipid A (GLA); QS-21 – a saponin extracted from the bark of the *Quillaja saponaria* tree, also known as the soap bark tree or Soapbark; resiquimod (TLR7/8 agonist), CDX-1401 – a fusion protein consisting of a fully human monoclonal antibody with specificity for the dendritic cell receptor DEC-205 linked to the NY-ESO-1 tumor antigen; Juvaris' Cationic Lipid-DNA Complex; Vaxfectin; and combinations thereof.

Polyinosinic-Polycytidylic acid (Poly IC) is a double-stranded RNA (dsRNA) that acts as a TLR3 agonist. To increase half-life, it has been stabilized with polylysine and carboxymethylcellulose as poly-ICLC. It has been used to induce interferon in cancer patients, with intravenous doses up to 300 μg/kg. Like poly-IC, poly-ICLC is a TLR3 agonist. TLR3 is expressed in the early endosome of myeloid DC; thus poly ICLC preferentially activates myeloid dendritic cells, thus favoring a Th1 cytotoxic T-cell response. Poly ICLC activates natural killer (NK) cells, induces cytolytic potential, and induces IFN-gamma from myeloid DC.

In some embodiments, the adjuvant is provided at about or at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 micrograms per dose or per kg in each dose. In some embodiments, the adjuvant is provided at least or about 0.1, 0.2, 0.3, 0.40, 0.50, 0.60, 0.70,

0.80, 0.90, 0.100, 1.10, 1.20, 1.30, 1.40, 1.50, 1.60, 1.70, 1.80, 1.90, 2.00, 2.10, 2.20, 2.30, 2.40, 2.50, 2.60, 2.70, 2.80, 2.90, 3.00, 3.10, 3.20, 3.30, 3.40, 3.50, 3.60, 3.70, 3.80, 3.90, 4.00, 4.10, 4.20, 4.30, 4.40, 4.50, 4.60, 4.70, 4.80, 4.90, 5.00, 5.10, 5.20, 5.30, 5.40, 5.50, 5.60, 5.70, 5.80, 5.90, 6.00, 6.10, 6.20, 6.30, 6.40, 6.50, 6.60, 6.70, 6.80, 6.90, 7.00, 7.10, 7.20, 7.30, 7.40, 7.50, 7.60, 7.70, 7.80, 7.90, 8.00, 8.10, 8.20, 8.30, 8.40, 8.50, 8.60, 8.70, 8.80, 8.90, 9.00, 9.10, 9.20, 9.30, 9.40, 9.50, 9.60, 9.70, 9.80, or 9.90 grams per dose or per kg in each dose. In some embodiments, the adjuvant is given at about or at least 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 500, 525, 550, 575, 600, 625, 675, 700, 725, 750, 775, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 endotoxin units ("EU") per dose.

The target peptide compositions of the presently disclosed subject matter can in some embodiments be provided with an administration of cyclophosamide around the time, (*e.g.*, about or at least 1, 2, 3, or 4 weeks or days before or after) the initial dose of a target peptide composition. An exemplary dose of cyclophosamide would in some embodiments be about or at least 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 mg/m^2/day over about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days.

The compositions of the presently disclosed subject matter can in some embodiments comprise the presently disclosed target peptides in the free form and/or in the form of a pharmaceutically acceptable salt.

As used herein, "a pharmaceutically acceptable salt" refers to a derivative of the disclosed target peptides wherein the target peptide is modified by making acid or base salts of the target peptide. For example, acid salts are prepared from the free base (typically wherein the neutral form of the drug has a neutral $--NH_2$ group) involving reaction with a suitable acid. Suitable acids for preparing acid salts include both organic acids such as but not limited to acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like, as well as inorganic acids such as but not limited to hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Conversely, basic salts of acid moieties which can be present on a target peptide are prepared using a pharmaceutically acceptable base such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, trimmethylamine or the like. By way of example and not limitation, the

compositions can in some embodiments comprise the target peptides as salts of acetic acid (acetates), ammonium, or hydrochloric acid (chlorides).

In some embodiments, a composition can include one or more sugars, sugar alcohols, amino acids such a glycine, arginine, glutaminic acid, and others as framework former. The sugars can be mono-, di- or trisaccharide. These sugars can be used alone, as well as in combination with sugar alcohols. Examples of sugars include glucose, mannose, galactose, fructose or sorbose as monosaccharides, sucrose, lactose, maltose or trehalose as disaccharides and raffinose as a trisaccharide. A sugar alcohol can be, for example, mannitose. In some embodiments, the composition comprises sucrose, lactose, maltose, trehalose, mannitol and/or sorbitol. In some embodiments, the composition comprises mannitol.

Furthermore, in some embodiments the presently disclosed compositions can include physiological well-tolerated excipients (*see e.g.*, the Rowe *et al.*, 2006), such as antioxidants like ascorbic acid or glutathione, preserving agents such as phenol, m-cresole, methyl- or propylparabene, chlorobutanol, thiomersal or benzalkoniumchloride, stabilizer, framework former such as sucrose, lactose, maltose, trehalose, mannitose, mannitol and/or sorbitol, mannitol and/or lactose and solubilizer such as polyethyleneglycols (PEG), *i.e.* PEG 3000, 3350, 4000, or 6000, or cyclodextrines, *i.e.* hydroxypropyle-β-cyclodextrine, sulfobutylethyl-β-cyclodextrine or γ-cyclodextrine, or dextranes or poloxaomers, *i.e.* poloxaomer 407, poloxamer 188, or TWEEN™20, TWEEN™ 80. In some embodiments, one or more well tolerated excipients can be included, selected from the group consisting of antioxidants, framework formers, and stabilizers.

In some embodiments, the pH for intravenous and intramuscular administration is selected from pH 2 to pH 12, while the pH for subcutaneous administration is selected from pH 2.7 to pH 9.0 as the rate of *in vivo* dilution is reduced resulting in more potential for irradiation at the injection site. (Strickley, 2004).

VII.C. Dosage

It is understood that a suitable dosage of a target peptide composition vaccine immunogen will depend upon the age, sex, health, and weight of the recipient, the kind of concurrent treatment, if any, the frequency of treatment, and the nature of the effect desired. However, a desired dosage can be tailored to the individual subject, as determined by the researcher or clinician. The total dose employed for any given treatment can typically be determined with respect to a standard reference dose based on the experience

of the researcher or clinician, such dose being administered either in a single treatment or in a series of doses, the success of which can depend on the production of a desired immunological result (*i.e.*, successful production of a T helper cell and/or CTL-mediated response to the target peptide immunogen composition, which response gives rise to the prevention and/or treatment desired). Thus, in some embodiments the overall administration schedule can be considered in determining the success of a course of treatment and not whether a single dose, given in isolation, would or would not produce the desired immunologically therapeutic result or effect. As such, a therapeutically effective amount (*i.e.*, that producing the desired T helper cell and/or CTL-mediated response) can in some embodiments depend on the antigenic composition of the vaccine used, the nature of the disease condition, the severity of the disease condition, the extent of any need to prevent such a condition where it has not already been detected, the manner of administration dictated by the situation requiring such administration, the weight and state of health of the individual receiving such administration, and/or the sound judgment of the clinician or researcher. Needless to say, the efficacy of administering additional doses and of increasing or decreasing the interval can be re-evaluated on a continuing basis, in view of the recipient's immunocompetence (for example, the level of T helper cell and/or CTL activity with respect to tumor-associated or tumor-specific antigens).

The concentration of the T helper or CTL stimulatory target peptides of the presently disclosed subject matter in pharmaceutical formulations are subject to wide variation, including anywhere from less than 0.01% by weight to as much as 50% or more. Factors such as volume and viscosity of the resulting composition can also be considered. The solvents, or diluents, used for such compositions can include one or more of water, phosphate buffered saline (PBS), saline itself, and/or other possible carriers and/or excipients. The immunogens of the presently disclosed subject matter can in some embodiments also be contained in artificially created structures such as liposomes, which structures can in some embodiments contain additional molecules, such as proteins or polysaccharides, inserted in the outer membranes of the structures and having the effect of targeting the liposomes to particular areas of the body, or to particular cells within a given organ or tissue. Such targeting molecules can in some embodiments be some type of immunoglobulin. Antibodies can work particularly well for targeting the liposomes to tumor cells.

Single i.d., i.m., s.c., i.p., and/or i.v. doses of *e.g.*, about 1 to 50 μg, 1 to 100 μg, 1 to 500 μg, 1 to 1000 μg, or about 1 to 50 mg, 1 to 100 mg, 1 to 500 mg, or 1 to 1000 mg of

a target peptide composition of the presently disclosed subject matter can in some embodiments be given and in some embodiments can depend from the respective compositions of target peptides with respect to total amount for all target peptides in the composition or alternatively for each individual target peptide in the composition. A single dose of a target peptide vaccine composition of the presently disclosed subject matter can in some embodiments have a target peptide amount (*e.g.*, total amount for all target peptides in the composition or alternatively for each individual target peptide in the composition) of about or at least 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, or 950 μg. Alternatively, a single dose of a target peptide composition of the presently disclosed subject matter can in some embodiments have a total target peptide amount (*e.g.*, total amount for all target peptides in the composition or alternatively for each individual target peptide in the composition) of about or at least 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, or 950 mg. In some embodiments, the target peptides of a composition of the presently disclosed subject matter are present in equal amounts of about 100 micrograms per dose in combination with an adjuvant peptide present in an amount of about 200 micrograms per dose.

In a single dose of the target peptide composition of the presently disclosed subject matter, the amount of each target peptide in the composition is in some embodiments equal or is in some embodiments substantially equal. Alternatively, the ratio of the target peptides present in the least amount relative to the target peptide present in the greatest amount is in some embodiments about or at least 1:1.25, 1:1.5, 1:1.75, 1:2.0, 1:2.25, 1:2.5, 1:2.75, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:30; 1:40, 1:50, 1:100, 1:200, 1:500, 1:1000, 1:5000; 1:10,000; or 1:100,000. Alternatively, the ratio of the target peptides present in the least amount relative to the target peptide present in the greatest amount is in some embodiments about or at least 1 or 2 to 25; 1 or 2 to 20; 1 or 2 to 15; 1 or 2 to 10; 1 to 3; 1 to 4; 1 to 5; 1 to 6; 1 to 7; 1 to 10; 2 to 3; 2 to 4; 2 to 5; 2 to 6; 2 to 7; 2 to 10; 3 to 4; 3 to 5; 3 to 6; 3 to 7; 3 to 10; 5 to 10; 10 to 15; 15 to 20; 20 to 25; 1 to 40; 1 to 30; 1 to 20; 1 to 15; 10 to 40; 10 to 30; 10 to 20; 10 to 15; 20 to 40; 20 to 30; or 20 to 25; 1 to 100; 25 to 100; 50 to 100; 75 to 100; 25 to 75, 25 to 50, or 50 to 75; 25 to 40; 25 to 50; 30 to 50; 30 to 40; or 30 to 75.

Single dosages can in some embodiments be given to a patient about or at least 1, 2, 3, 4, or 5 times per day. Single dosages can in some embodiments be given to a patient about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 36, 48, 60, or 72 hours subsequent to a previous dose.

Single dosages can in some embodiments be given to a patient about or at least 1, 2, 3, 4, 5, 6, or 7 times per week or every other, third, fourth, or fifth day. Single doses can in some embodiments also be given every week, every other week, or only during 1, 2, or 3 weeks per month. A course of treatment can in some embodiments last about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months.

In some embodiments, single dosages of the compositions of the presently disclosed subject matter are provided to a patient in at least two phases, *e.g.*, during an initial phase and then a subsequent phase. An initial phase can in some embodiments be about or at least 1, 2, 3, 4, 5, or 6 weeks in length. The subsequent phase can in some embodiments last at least or about 1, 2, 3, 4, 5, 6, 7, or 8 times as long as the initial phase. The initial phase can in some embodiments be separated from the subsequent phase by about or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks or months.

The target peptide composition dosage during the subsequent phase can in some embodiments be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 times greater than during the initial phase. The target peptide composition dosage during the subsequent phase can in some embodiments be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 times lower than during the initial phase.

In some embodiments, the initial phase is about three weeks and the second phase is about 9 weeks. In some embodiments, the target peptide compositions would be administered to the patient on or about days 1, 8, 15, 36, 57, and 78.

VII.D. Kits and Storage

In some embodiments, the presently disclosed subject matter provides a kit. In some embodiments the kit comprises (a) a container that contains at least one target peptide composition as described above in solution or in lyophilized form; (b) optionally, a second container containing a diluent or reconstituting solution for the lyophilized formulation; and (c) also optionally, instructions for (i) use of the solution; and/or (ii) reconstitution and/or use of the lyophilized formulation. The kit can in some embodiments further comprise one or more of (iii) a buffer, (iv) a diluent, (v) a filter, (vi) a needle, and/or (v) a syringe. In some embodiments, the container is selected from the group

consisting of a bottle, a vial, a syringe, a test tube, and a multi-use container. In some embodiments, the target peptide composition is lyophilized.

The kits can in some embodiments contain exactly, about, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 45, 46, 47, 48, 49, 50, 51, or more target peptide-containing compositions. Each composition in the kit can in some embodiments be administered at the same time or at different times to a subject.

In some embodiments, the kits can comprise a lyophilized formulation of the presently disclosed compositions and/or vaccines in a suitable container and instructions for its reconstitution and/or use. Suitable containers include, for example, bottles, vials (*e.g.* dual chamber vials), syringes (such as dual chamber syringes), and test tubes. The container can in some embodiments be formed from a variety of materials such as glass or plastic. In some embodiments, the kit and/or container include instructions on or associated with the container that indicate directions for reconstitution and/or use. For example, the label can in some embodiments indicate that the lyophilized formulation is to be reconstituted to target peptide concentrations as described above. The label can in some embodiments further indicate that the formulation is useful or intended for subcutaneous administration. Lyophilized and liquid formulations are in some embodiments stored at -20◦C to -80◦C.

The container holding the target peptide composition(s) can in some embodiments be a multi-use vial, which allows for repeat administrations (*e.g.*, from 2-6 administrations) of the reconstituted formulation. The kit can in some embodiments further comprise a second container comprising a suitable diluent such as, but not limited to a sodium bicarbonate solution.

In some embodiments, upon mixing of the diluent and the lyophilized formulation, the final peptide concentration in the reconstituted formulation is at least or about 0.15, 0.20, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.50, 3.75, 4.0, 4.25, 4.5, 4.75, 5.0, 6.0, 7.0, 8.0, 9.0, or 10 mg/mL/target peptide. In some embodiments, upon mixing of the diluent and the lyophilized formulation, the final peptide concentration in the reconstituted formulation is at least or about 0.15, 0.20, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.50, 3.75, 4.0, 4.25, 4.5, 4.75, 5.0, 6.0, 7.0, 8.0, 9.0 or 10 μg/mL/target peptide.

The kit can in some embodiments further comprise other materials desirable from a commercial and user standpoint, including but not limited to other buffers, diluents, filters, needles, syringes, and/or package inserts with instructions for use.

The kits can in some embodiments have a single container that comprises the formulation of the target peptide compositions with or without other components (*e.g.*, other compounds or compositions of these other compounds) or can in some embodiments have a distinct container for each component.

Additionally, the kits can in some embodiments comprise a formulation of the presently disclosed target peptide compositions and/or vaccines packaged for use in combination with the co-administration of a second compound such as but not limited to adjuvants (*e.g.* imiquimod), a chemotherapeutic agent, a natural product, a hormone or antagonist, an anti-angiogenesis agent or inhibitor, an apoptosis-inducing agent, or a chelator or a composition thereof. The components of the kit can in some embodiments be pre-complexed or each component can in some embodiments be in a separate distinct container prior to administration to a patient. The components of the kit can in some embodiments be provided in one or more liquid solutions. In some embodiments, the liquid solution is an aqueous solution. In some embodiments, the liquid solution is a sterile aqueous solution. The components of the kit can in some embodiments also be provided as solids, which in some embodiments are converted into liquids by addition of suitable solvents, which can in some embodiments be provided in another distinct container.

The container of a therapeutic kit can in some embodiments be a vial, a test tube, a flask, a bottle, a syringe, or any other article suitable to enclose a solid or liquid. In some embodiments, when there is more than one component, the kit can contain a second vial and/or other container, which allows for separate dosing. The kit can in some embodiments also contain another container for a pharmaceutically acceptable liquid. In some embodiments, a therapeutic kit contains an apparatus (*e.g.*, one or more needles, syringes, eye droppers, pipette, etc.) that facilitates administration of the agents of the disclosure that are components of the present kit.

VII.E. Markers for Efficacy

When administered to a patient, the vaccine compositions of the presently disclosed subject matter are envisioned to have certain physiological effects, including but not limited to the induction of a T cell mediated immune response.

VII.E.1 Immunohistochemistry, Immunofluorescence, Western Blots, and Flow Cytometry

Validation and testing of antibodies for characterization of cellular and molecular features of lymphoid neogenesis has been performed. Commercially available antibodies for use in immunohistochemistry (IHC), immunofluorescence (IF), flow cytometry (FC), and western blot (WB) can in some embodiments be employed. In some embodiments, such techniques can be employed to analyze patient samples, *e.g.*, formalin-fixed, paraffin-embedded tissue samples, for CD1a, S100, CD83, DC-LAMP, CD3, CD4, CD8, CD20, CD45, CD79a, PNAd, TNFalpha, LIGHT, CCL19, CCL21, CXCL12, TLR4, TLR7, FoxP3, PD-1 and Ki67 expression. In some embodiments, flow cytometry is used to determine CD3, CD4, CD8, CD13, CD14, CD16, CD19, CD45RA, CD45RO, CD56, CD62L, CD27, CD28, CCR7, FoxP3 (intracellular), and MHC-peptide tetramers for I MHC associated (phospho)-peptides. In some embodiments, positive control tissue selected from among normal human peripheral blood lymphocytes (PBL), PBL activated with CD3/CD28 beads (activated PBL), human lymph node tissue from non-HCC patients (LN), and inflamed human tissue from a surgical specimen of Crohn's disease (Crohn's) can be employed.

VII.E.2. ELISpot Assay

In some embodiments, vaccination site infiltrating lymphocytes and lymphocytes from the sentinel immunized nod (SIN) and vaccine site can be evaluated by ELISpot. ELISpot permits the direct counting of T-cells reacting to antigen by production of INFγ. Peripheral blood lymphocytes can be evaluated by ELISpot assay for the number of peptide-reactive T-cells. Vaccine site infiltrating lymphocytes and SIN lymphocytes can be compared to those in peripheral blood. It is envisioned that positive results of the ELISpot assay correlate with increased patient progression free survival. Progression free survival is in some embodiments defined as the time from start of treatment until death from any cause or date of last follow up.

VII.E.3. Tetramer Assay

Peripheral blood lymphocytes and lymphocytes from the SIN and vaccine site can be evaluated by flow cytometry after incubation with MHC-peptide tetramers for the number of peptide-reactive T-cells.

VII.E.4. Proliferation Assay/Cytokine Analysis

Peripheral blood mononuclear cells (PBMC), vaccine-site inflammatory cells, and lymphocytes from the SIN from patients can in some embodiments be evaluated for CD4

T cell reactivity to, *e.g.*, tetanus helper peptide mixture, using a ^{3}H-thymidine uptake assay. Additionally, Th1 (IL-2, IFN-gamma, TNFa), Th2 (IL-4, IL-5, IL-10), Th17 (IL-17, and IL23), and T-reg (TGF-beta) cytokines in media from 48 hours in that proliferation assay can be employed to determine if the microenvironment supports generation of Th1, Th2, Th17, and/or T-reg responses. In some embodiments, two peptides are used as negative controls: a tetanus peptide and the Pan DR T helper epitopes (PADRE) peptide (AK(X)VAAWTLKAA; SEQ ID NO: 500).

VII.E.5. Evaluation of Tumors

In some embodiments tumor tissue collected prior to treatment or at the time of progression can be evaluated by routine histology and immunohistochemistry. Alternatively or in addition, *in vitro* evaluations of tumor tissue and tumor infiltrating lymphocytes can be completed.

VII.E.6. Studies of Homing Receptor Expression

Patient samples can in some embodiments be studied for T cell homing receptors induced by vaccination the compositions of the presently disclosed subject matter. These include, but are not limited to, integrins (including alphaE-beta7, alpha1-beta1, alpha4-beta1), chemokine receptors (including CXCR3), and selectin ligands (including CLA, PSL) on lymphocytes, and their ligands in the vaccine sites and SIN. These can be assayed by immunohistochemistry, flow cytometry or other techniques.

VII.E.7. Studies of Gene and Protein Expression

Differences in gene expression and/or for differences in panels of proteins can in some embodiments be assayed by high-throughput screening assays (*e.g.* nucleic acid chips, protein arrays, etc.) in the vaccine sites and sentinel immunized nodes.

VIII. Antibodies Including Antibody-Like Molecules

In some embodiments, the present disclosure provides antibodies and antibody-like molecules (*e.g.* T cell receptors) that specifically bind to the target peptides (e.g., phosphopeptides) disclosed herein, or to complexes of an MHC molecule (e.g., a class I MHC fmolecule) and the peptides disclosed herein. In some embodiments, the antibodies and antibody-like molecules (*e.g.* T cell receptors) specifically bind to complexes of phosphopeptides and corresponding MHC alleles as set forth in Tables 2-14.

Antibodies and antibody-like molecules (*e.g.* T cell receptors) specific for target peptides or target peptide/MHC complexes are, for example, useful, *inter alia*, for analyzing tissue to determine the pathological nature of tumor margins and/or can be employed in some embodiments as therapeutics. Alternatively, such molecules can in

some embodiments be employed as therapeutics targeting cells, *e.g.*, tumor cells, which display target peptides on their surface. In some embodiments, the antibodies and antibody-like molecules bind the target peptides or target peptide-MHC complex specifically and do not substantially cross react with non-phosphorylated native peptides.

As used herein, "antibody" and "antibody peptide(s)" refer to intact antibodies, antibody-like molecules, and binding fragments thereof that compete with intact antibodies for specific binding. Binding fragments are in some embodiments produced by recombinant DNA techniques or in some embodiments by enzymatic or chemical cleavage of intact antibodies. Binding fragments include Fab, Fab', $F(ab')_2$, Fv, and single-chain antibodies. An antibody other than a "bispecific" or "bifunctional" antibody is understood to have each of its binding sites identical. An antibody in some embodiments substantially inhibits adhesion of a receptor to a counterreceptor when an excess of antibody reduces the quantity of receptor bound to counterreceptor by at least about 20%, 40%, 60%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater than 99% as measured, for example, in an *in vitro* competitive binding assay.

The term "MHC" as used herein refers to the Major Histocompability Complex, which is defined as a set of gene loci specifying major histocompatibility antigens. The term "HLA" as used herein refers to Human Leukocyte Antigens, which are defined as the histocompatibility antigens found in humans. As used herein, "HLA" is the human form of "MHC".

The terms "MHC light chain" and "MHC heavy chain" as used herein refer to portions of MHC molecules. Structurally, class I molecules are heterodimers comprised of two non-covalently bound polypeptide chains, a larger "heavy" chain (α) and a smaller "light" chain (β-2-microglobulin or β2m). The polymorphic, polygenic heavy chain (45 kDa), encoded within the MHC on chromosome six, is subdivided into three extracellular domains (designated 1, 2, and 3), one intracellular domain, and one transmembrane domain. The two outermost extracellular domains, 1 and 2, together form the groove that binds antigenic peptide. Thus, interaction with the TCR occurs at this region of the protein. The 3 domain of the molecule contains the recognition site for the CD8 protein on the CTL; this interaction serves to stabilize the contact between the T cell and the APC. The invariant light chain (12 kDa), encoded outside the MHC on chromosome 15, consists of a single, extracellular polypeptide. The terms "MHC light chain", "β2-microglobulin", and "β2m" are used interchangeably herein.

The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. An antibody or antibody like molecule is said to "specifically" bind an antigen when the dissociation constant is in some embodiments less than 1 μM, in some embodiments less than 100 nM, and in some embodiments less than 10 nM.

The term "antibody" is used in the broadest sense, and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments (*e.g.*, Fab, F(ab')$_2$ and Fv), as well as "antibody-like molecules" so long as they exhibit the desired biological activity. Antibodies (Abs) and immunoglobulins (Igs) are glycoproteins having the same structural characteristics. The term is also meant to encompass "antibody like molecules" and other members of the immunoglobulin superfamily, *e.g.*, T-cell receptors, MHC molecules, containing *e.g.*, an antigen-binding regions and/or variable regions, *e.g.*, complementary determining regions (CDRs) which specifically bind the target peptides disclosed herein.

In some embodiments, antibodies and antibody-like molecules bind to the target peptides of the presently disclosed subject matter but do not substantially and/or specifically cross react with the same peptide in a modified form. *See e.g.*, U.S. Patent Application Publication No. 2009/0226474, which is incorporated by reference.

The presently disclosed subject matter also includes antibodies that recognize target peptides associated with a tumorigenic or disease state, wherein the peptides are displayed in the context of HLA molecules. These antibodies typically mimic the specificity of a T cell receptor (TCR) but can in some embodiments have higher binding affinity such that the molecules can be employed as therapeutic, diagnostic, and/or research reagents. Methods of producing a T-cell receptor mimic of the presently disclosed subject matter include identifying a target peptide of interest (*e.g.*, a phosphopeptide), wherein the target peptide of interest comprises an amino acid sequence as set forth in any of SEQ ID NOs: 1-448 and 502-529 (*e.g.*, a phosphopeptide as set forth in Tables 2-14 herein). Then, an immunogen comprising at least one target peptide/MHC complex is formed. An effective amount of the immunogen is then administered to a host for eliciting an immune response, and serum collected from the host is assayed to determine if desired antibodies that recognize a three-dimensional presentation of the target peptide in the

binding groove of the MHC molecule are being produced. The desired antibodies can differentiate the target peptide/MHC complex from the MHC molecule alone, the target peptide alone, and a complex of MHC and irrelevant target peptide. Finally, in some embodiments the desired antibodies are isolated.

The term "antibody" also encompasses soluble T cell receptors (TCR) which are stable at low concentrations and which can recognize MHC-peptide complexes. *See e.g.*, U.S. Patent Application Publication No. 2002/0119149, which is incorporated by reference. Such soluble TCRs might for example be conjugated to immunostimulatory peptides and/or proteins or moieties, such as CD3 agonists (anti-CD3 antibody), for example. The CD3 antigen is present on mature human T cells, thymocytes, and a subset of natural killer cells. It is associated with the TCR and is responsible for the signal transduction of the TCR.

Antibodies specific for the human CD3 antigen are well-known. One such antibody is the murine monoclonal antibody OKT3 which was the first monoclonal antibody approved by the FDA. OKT3 is reported to be a potent T cell mitogen (*see e.g.*, Van Wauve, 1980; U.S. Patent No. 4,361,539) and a potent T cell killer (Wong, 1990. Other antibodies specific for the CD3 antigen have also been reported (*see e.g.*, PCT International Patent Application Publication No. WO 2004/0106380; U.S. Patent Application Publication No. 2004/0202657; U.S. Patent No. 6,750,325; U.S. Patent No. 6,706,265; GB 2249310A; Clark *et al.*, 1989; U.S. Patent No. 5,968,509; and U.S. Patent Application Publication No. 2009/0117102). ImmTACs (Immunocore Limited, Milton Park, Abington, Oxon, United Kingdom) are innovative bifunctional proteins that combine high-affinity monoclonal T cell receptor (mTCR) targeting technology with a clinically-validated, highly potent therapeutic mechanism of action (Anti-CD3 scFv).

Native antibodies and immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond. The number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to

form an interface between the light and heavy chain variable domains (Chothia *et al.*, 1985; Novotny & Haber, 1985).

An "isolated" antibody is one which has been separated, identified, and/or recovered from a component of the environment in which it was produced. Contaminant components of its production environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and can include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, the antibody is purified as measurable by at least one of the following three different methods: 1) to in some embodiments greater than 50% by weight of antibody as determined by the Lowry method, such as but not limited to in some embodiments greater than 75% by weight, in some embodiments greater than 85% by weight, in some embodiments greater than 95% by weight, in some embodiments greater than 99% by weight; 2) to a degree sufficient to obtain at least 10 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequentator, such as at least 15 residues of sequence; or 3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomasie blue or, in some embodiments, silver stain. Isolated antibodies include the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment is not present. In some embodiments, however, isolated antibodies are prepared by a method that includes at least one purification step.

The terms "antibody mutant", "antibody variant", and "antibody derivative" refer to an amino acid sequence variant of an antibody wherein one or more of the amino acid residues of a reference antibody has been modified (*e.g.*, substituted, deleted, chemically modified, etc.). Such mutants necessarily have less than 100% sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the reference antibody. The resultant sequence identity or similarity between the modified antibody and the reference antibody is thus in some embodiments at least 80%, in some embodiments at least 85%, in some embodiments at least 90%, in some embodiments at least 95%, in some embodiments at least 97%, and in some embodiments at least 99%.

The term "variable" in the context of variable domain of antibodies, refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen(s). However, the variability is not evenly distributed through the variable domains of antibodies. It is concentrated in three segments called

complementarity determining regions (CDRs) also known as hypervariable regions both in the light chain and the heavy chain variable domains. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (Kabat *et al.*, 1987); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Chothia *et al.*, 1989). The more highly conserved portions of variable domains are called the framework (FR) regions. The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies (*see* Kabat *et al.*, 1987). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector function, such as participation of the antibody in antibody-dependent cellular toxicity.

The term "antibody fragment" refers to a portion of a full-length antibody, generally the antigen binding or variable region. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments. Papain digestion of antibodies produces two identical antigen binding fragments, called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment, so-called for its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen binding fragments which are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). As used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and $F(ab')_2$ fragments.

An "Fv" fragment is the minimum antibody fragment which contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (V_H-V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment, also designated as F(ab), also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains have a free thiol group. F(ab') fragments are produced by cleavage of the disulfide bond at the hinge cysteines of the $F(ab')_2$ pepsin digestion product. Additional chemical couplings of antibody fragments are known to those of ordinary skill in the art.

The light chains of antibodies (immunoglobulin) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino sequences of their constant domain.

Depending on the amino acid sequences of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are at least five (5) major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these can be further divided into subclasses (isotypes), *e.g.*, IgG_1, IgG_2, IgG_3, and IgG_4; IgA_1 and IgA_2. The heavy chains constant domains that correspond to the different classes of immunoglobulins are called alpha (α), delta (Δ), epsilon (ε), gamma (γ), and mu (μ), respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well-known.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that can be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, monoclonal antibodies can be advantageous in that they can be synthesized in hybridoma culture, uncontaminated by other immunoglobulins.

The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the presently disclosed subject matter can in some embodiments be made by the hybridoma method first described by Kohler & Milstein, 1975, or can in some embodiments be made by recombinant methods, *e.g.*, as described in U.S. Patent No. 4,816,567. The monoclonal antibodies for use with the presently disclosed subject matter can in some embodiments also be isolated from

phage antibody libraries using the techniques described in Clackson *et al.*, 1991 or in Marks *et al.*, 1991.

Utilization of the monoclonal antibodies of the presently disclosed subject matter can in some embodiments require administration of such or similar monoclonal antibody to a subject, such as a human. However, when the monoclonal antibodies are produced in a non-human animal, such as a rodent, administration of such antibodies to a human patient will normally elicit an immune response, wherein the immune response is directed towards the antibodies themselves. Such reactions limit the duration and effectiveness of such a therapy. In order to overcome such problem, the monoclonal antibodies of the presently disclosed subject matter can be "humanized": that is, the antibodies can be engineered such that antigenic portions thereof are removed and like portions of a human antibody are substituted therefor, while the antibodies' affinity for specific peptide/MHC complexes is retained. This engineering can in some embodiments only involve a few amino acids, or can in some embodiments include entire framework regions of the antibody, leaving only the complementarity determining regions of the antibody intact. Several methods for humanizing antibodies are known in the art and are disclosed, for example, in U.S. Patent Nos. 4,816,567; 5,712,120; 5,861,155; 5,869,619; 6,054,927; and 6,180,370; the entire content of each of which is hereby expressly incorporated herein by reference in its entirety.

Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. In some embodiments, humanization can be performed following the method of Winter and co-workers (*see e.g.*, Jones *et al.*, 1986; Riechmann *et al.*, 1988; Verhoeyen *et al.*, 1988) by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. *See also* U.S. Patent No. 5,225,539. In some embodiments, F_V framework residues of a human immunoglobulin are replaced by corresponding non-human residues.

Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, a humanized antibody comprises substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a

human immunoglobulin consensus sequence. The humanized antibody optimally can in some embodiments also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. *See e.g.*, Jones *et al.*, 1986; Riechmann *et al.*, 1988; Presta, 1992.

Many articles relating to the generation or use of humanized antibodies teach useful examples of protocols that can be utilized with the presently disclosed subject matter, such as but not limited to Shinkura *et al.*, 1998; Yenari *et al.*, 1998; Richards *et al.*, 1999; Morales *et al.*, 2000; Mihara *et al.*, 2001; Sandborn *et al.*, 2001; and Yenari *et al.*, 2001, all of which are expressly incorporated in their entireties by reference. For example, a treatment protocol that can be utilized in such a method includes a single dose, generally administered intravenously, of 10-20 mg of humanized mAb per kg (Sandborn, *et al.*, 2001). In some embodiments, alternative dosing patterns can be appropriate, such as but not limited to the use of three infusions, administered once every two weeks, of 800 to 1600 mg or even higher amounts of humanized mAb (Richards *et al.*, 1999, *op. cit.*). However, it is to be understood that the presently disclosed subject matter is not limited to the treatment protocols described above, and other treatment protocols that are known to a person of ordinary skill in the art can be utilized in the methods of the presently disclosed subject matter.

The presently disclosed and claimed subject matter further includes in some embodiments fully human monoclonal antibodies against specific target peptide/MHC complexes. Fully human antibodies essentially relate to antibody molecules in which the entire sequence of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are referred to herein as "human antibodies" or "fully human antibodies". Human monoclonal antibodies can be prepared by the trioma technique; the human B-cell hybridoma technique (*see* Kozbor *et al.*, 1983), and the EBV hybridoma technique to produce human monoclonal antibodies (*see* Cole *et al.*, 1985). Human monoclonal antibodies can in some embodiments be utilized in the practice of the presently disclosed subject matter and can in some embodiments be produced by using human hybridomas (*see* Cote *et al.*, 1983)) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*see* Cole *et al.*, 1985).

In addition, human antibodies can also be produced using additional techniques, including but not limited to phage display libraries (Hoogenboom *et al.*, 1991; Marks *et al.*, 1991). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous

immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; and in Marks *et al.*, 1992; Lonberg *et al.*, 1994; Lonberg & Huszar, 1995; Fishwild *et al.*, 1996; Neuberger, 1996.

Human antibodies can in some embodiments additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. *See* PCT International Patent Application Publication No. WO 1994/02602). Typically, the endogenous genes encoding the heavy and light immunoglobulin chains in the non-human host are incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal that provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications.

A non-limiting example of such a nonhuman animal is a mouse, and is termed the XENOMOUSE™ as disclosed in PCT International Patent Application Publication Nos. WO 1996/33735 and WO 1996/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a non-human host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598, incorporated herein by reference). It can be obtained by a method including deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from

the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

An exemplary method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771 incorporated herein by reference). It includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

The antigen target peptides are known to be expressed on a variety of cancer cell types. Thus, antibodies and antibody-like molecules can be used where appropriate, in treating, diagnosing, vaccinating, preventing, retarding, and/or attenuating HCC, melanoma, ovarian cancer, breast cancer, colorectal cancer, squamous carcinoma of the lung, sarcoma, renal cell carcinoma, pancreatic carcinomas, squamous tumors of the head and neck, leukemia, brain cancer, liver cancer, prostate cancer, ovarian cancer, and cervical cancer.

Antibodies generated with specificity for the antigen target peptides can be used to detect the corresponding target peptides in biological samples. The biological sample could come from an individual who is suspected of having cancer and thus detection would serve to diagnose the cancer. Alternatively, the biological sample can in some embodiments come from an individual known to have cancer, and detection of the antigen target peptides would serve as an indicator of disease prognosis, cancer characterization, or treatment efficacy. Appropriate immunoassays are well-known in the art and include, but are not limited to, immunohistochemistry, flow cytometry, radioimmunoassay, western blotting, and ELISA. Biological samples suitable for such testing include, but are not limited to, cells, tissue biopsy specimens, whole blood, plasma, serum, sputum, cerebrospinal fluid, pleural fluid, and urine. Antigens recognized by T cells, whether helper T lymphocytes or CTL, are not recognized as intact proteins, but rather as small peptides that associate with class I or class II MHC proteins on the surface of cells. During the course of a naturally occurring immune response antigens that are recognized in association with class II MHC molecules on antigen presenting cells are acquired from outside the cell, internalized, and processed into small peptides that associate with the class II MHC molecules. Conversely, the antigens that give rise to proteins that are recognized in association with class I MHC molecules are generally proteins made within

the cells, and these antigens are processed and associate with class I MHC molecules. It is now well-known that the peptides that associate with a given class I or class II MHC molecule are characterized as having a common binding motif, and the binding motifs for a large number of different class I and II MHC molecules have been determined. It is also well-known that synthetic peptides can be made which correspond to the sequence of a given antigen and which contain the binding motif for a given class I or II MHC molecule. These peptides can then be added to appropriate antigen presenting cells, and the antigen presenting cells can be used to stimulate a T helper cell or CTL response either *in vitro* or *in vivo*. The binding motifs, methods for synthesizing the peptides, and methods for stimulating a T helper cell or CTL response are all well-known and readily available.

As used herein, the terms "T cell receptor" and "TCR" are used interchangeably and refer to full length heterodimeric αβ or γδ TCRs, antigen-binding fragments of TCRs, or molecules comprising TCR CDRs or variable regions. Examples of TCRs include, but are not limited to, full-length TCRs, antigen-binding fragments of TCRs, soluble TCRs lacking transmembrane and cytoplasmic regions, single-chain TCRs containing variable regions of TCRs attached by a flexible linker, TCR chains linked by an engineered disulfide bond, monospecific TCRs, multi-specific TCRs (including bispecific TCRs), TCR fusions, human TCRs, humanized TCRs, chimeric TCRs, recombinantly produced TCRs, and synthetic TCRs. The term encompasses wild-type TCRs and genetically engineered TCRs (*e.g.*, a chimeric TCR comprising a chimeric TCR chain which includes a first portion from a TCR of a first species and a second portion from a TCR of a second species).

As used herein, the term "TCR variable region" is understood to encompass amino acids of a given TCR which are not included within the non-variable region as encoded by the TRAC gene for TCR α chains and either the TRBC1 or TRBC2 genes for TCR β chains. In some embodiments, a TCR variable region encompasses all amino acids of a given TCR which are encoded by a TRAV gene or a TRAJ gene for a TCR α chain or a TRBV gene, a TRBD gene, or a TRBJ gene for a TCR β chain (*see e.g.*, LeFranc & LeFranc, 2001, which is incorporated by reference herein in its entirety).

As used herein, the term "constant region" with respect to a TCR refers to the extracellular portion of a TCR that is encoded by the TRAC gene for TCR α chains and either the TRBC1 or TRBC2 genes for TCR β chains. The term constant region does not include a TCR variable region encoded by a TRAV gene or a TRAJ gene for a TCR α

chain or a TRBV gene, a TRBD gene, or a TRBJ gene for a TCR β chain (*see e.g.*, LeFranc & LeFranc, 2001, which is incorporated by reference herein in its entirety).

Kits can in some embodiments be composed for help in diagnosis, monitoring, and/or prognosis. The kits are to facilitate the detecting and/or measuring of cancer-specific target peptides or proteins. Such kits can in some embodiments contain in a single or divided container, a molecule comprising an antigen-binding region. Such molecules can in some embodiments be antibodies and/or antibody-like molecules. Additional components that can be included in the kit include, for example, solid supports, detection reagents, secondary antibodies, instructions for practicing, vessels for running assays, gels, control samples, and the like. The antibody and/or antibody-like molecules can in some embodiments be directly or indirectly labeled, as an option.

Alternatively or in addition, the antibody or antibody-like molecules specific for target peptides and/or target peptide/MHC complexes can in some embodiments be conjugated to therapeutic agents. Exemplary therapeutic agents include:

Alkylating Agents: Alkylating agents are drugs that directly interact with genomic DNA to prevent cells from proliferating. This category of chemotherapeutic drugs represents agents that affect all phases of the cell cycle, that is, they are not phase-specific. An alkylating agent can in some embodiments include, but is not limited to, a nitrogen mustard, an ethylenimene, a methylmelamine, an alkyl sulfonate, a nitrosourea or a triazines. They include but are not limited to busulfan, chlorambucil, cisplatin, cyclophosphamide (cytoxan), dacarbazine, ifosfamide, mechlorethamine (mustargen), and melphalan.

Antimetabolites: Antimetabolites disrupt DNA and RNA synthesis. Unlike alkylating agents, they specifically influence the cell cycle during S phase. Antimetabolites can be differentiated into various categories, such as folic acid analogs, pyrimidine analogs and purine analogs and related inhibitory compounds. Antimetabolites include but are not limited to 5-fluorouracil (5-FU), cytarabine (Ara-C), fludarabine, gemcitabine, and methotrexate.

Natural Products: Natural products generally refer to compounds originally isolated from a natural source, and identified as having a pharmacological activity. Such compounds, as well as analogs and derivatives thereof, can in some embodiments be isolated from a natural source, chemically synthesized or recombinantly produced by any technique known to those of skill in the art. Natural products include such categories as mitotic inhibitors, antitumor antibiotics, enzymes and biological response modifiers.

Mitotic inhibitors include plant alkaloids and other natural agents that can inhibit either protein synthesis required for cell division or mitosis. They operate during a specific phase during the cell cycle. Mitotic inhibitors include, for example, docetaxel, etoposide (VP16), teniposide, paclitaxel, taxol, vinblastine, vincristine, and vinorelbine.

Taxoids are a class of related compounds isolated from the bark of the ash tree, *Taxus brevifolia*. Taxoids include, but are not limited to, compounds such as docetaxel and paclitaxel. Paclitaxel binds to tubulin (at a site distinct from that used by the vinca alkaloids) and promotes the assembly of microtubules.

Vinca alkaloids are a type of plant alkaloid identified to have pharmaceutical activity. They include such compounds as vinblastine (VLB) and vincristine.

Antibiotics: Certain antibiotics have both antimicrobial and cytotoxic activity. These drugs can also interfere with DNA by chemically inhibiting enzymes and mitosis or altering cellular membranes. These agents are typically not phase-specific so they work in all phases of the cell cycle. Examples of cytotoxic antibiotics include but are not limited to bleomycin, dactinomycin, daunorubicin, doxorubicin (Adriamycin), plicamycin (mithramycin), and idarubicin.

Miscellaneous Agents: Miscellaneous cytotoxic agents that do not fall into the previous categories include but are not limited to platinum coordination complexes, anthracenediones, substituted ureas, methyl hydrazine derivatives, amsacrine, L-asparaginase, and tretinoin. Platinum coordination complexes include such compounds as carboplatin and cisplatin (cis-DDP). An exemplary anthracenedione is mitoxantrone. An exemplary substituted urea is hydroxyurea. An exemplary methyl hydrazine derivative is procarbazine (N-methylhydrazine, MIH). These examples are not limiting and it is contemplated that any known cytotoxic, cytostatic, and/or cytocidal agent can be conjugated or otherwise attached to targeting peptides and administered to a targeted organ, tissue, and/or cell type within the scope of the presently disclosed subject matter.

Chemotherapeutic (cytotoxic) agents include but are not limited to 5-fluorouracil, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin (CDDP), cyclophosphamide, dactinomycin, daunorubicin, doxorubicin, estrogen receptor binding agents, etoposide (VP16), farnesyl-protein transferase inhibitors, gemcitabine, ifosfamide, mechlorethamine, melphalan, mitomycin, navelbine, nitrosurea, plicomycin, procarbazine, raioxifene, tamoxifen, taxol, temazolomide (an aqueous form of DTIC), transplatinum, vinblastine and methotrexate, vincristine, or any analog or derivative variant of the foregoing. Most chemotherapeutic agents fall into the categories of alkylating agents,

antimetabolites, antitumor antibiotics, corticosteroid hormones, mitotic inhibitors, and nitrosoureas, hormone agents, miscellaneous agents, and any analog or derivative variant thereof.

The peptides identified and tested thus far in peptide-based vaccine approaches have generally fallen into one of three categories: 1) mutated on individual tumors, and thus not displayed on a broad cross section of tumors from different patients; 2) derived from unmutated tissue-specific proteins, and thus compromised by mechanisms of self-tolerance; and 3) expressed in subsets of cancer cells and normal testes.

Antigens linked to transformation or oncogenic processes are of primary interest for immunotherapeutic development based on the hypothesis that tumor escape through mutation of these proteins can be more difficult without compromising tumor growth or metastatic potential.

The target peptides of the presently disclosed subject matter are unique in that the identified target peptides are modified by intracellular modification. This modification is of particular relevance because it is associated with a variety of cellular control processes, some of which are dysregulated in cancer cells. For example, the source proteins for class I MHC-associated phosphopeptides are often known phosphoproteins, supporting the idea that the phosphopeptides are processed from folded proteins participating in signaling pathways.

Although not wishing to be bound by any particular theory, it is envisioned that the target peptides of the presently disclosed subject matter are unexpectedly superior to known tumor-associated antigen-derived peptides for use in immunotherapy because: 1) they only displayed on the surface of cells in which intracellular phosphorylation is dysregulated, *i.e.*, cancer cells, and not normal thymus cells, and thus they are not are not compromised by self-tolerance (as opposed to TAA which are associated with overexpression or otherwise expressed on non-mutated cells); and/or 2) they identify a cell displaying them on their surface as having dysregulated phosphorylation. Thus, post-translationally-modified phosphopeptides that are differentially displayed on cancer cells and derived from source proteins objectively linked to cellular transformation and metastasis allow for more extensive anti-tumor responses to be elicited following vaccination. Target peptides are, therefore, better immunogens in peptide-based vaccines, as target peptides are derived from proteins involved with cellular growth control, survival, or metastasis and alterations in these proteins as a mechanism of immune escape can interfere with the malignant phenotype of tumors.

As such, the presently disclosed subject matter also relates in some embodiments to methods for identifying target peptides for use in immunotherapy which are displayed on transformed cells but are not substantially expressed on normal tissue in general or in the thymus in particular. In some embodiments, target peptides bind the MHC class I molecule more tightly than their non-phosphorylated native counterparts. Moreover, such target peptides can in some embodiments have additional binding strength by having amino acid substitutions at certain anchor positions. In some embodiments, such modified target peptides can remain cross-reactive with TCRs specific for native target peptide MHC complexes. Additionally, it is envisioned that the target peptides associated with proteins involved in intracellular signaling cascades or cycle regulation are of particular interest for use in immunotherapy. In some cases, the TCR binding can specifically react with the phosphate groups on the target peptide being displayed on an MHC class I molecule.

In some embodiments, the method of screening target peptides for use in immunotherapy, *e.g.*, in adaptive cell therapy or in a vaccine, involves determining whether the candidate target peptides are capable of inducing a memory T cell response. The contemplated screening methods can include providing target peptides, *e.g.*, those disclosed herein or those to be identified in the future, to a healthy volunteer and determining the extent to which a target peptide-specific T cell response is observed. In some embodiments, the extent to which the T cell response is a memory T cell response is also determined. In some embodiments, it is determined the extent to which a T_{CM} response is elicited, *e.g.*, relative to other T cell types. In some embodiments, those target peptides which are capable of inducing a memory T cell response in health and/or diseased patients are selected for inclusion in the therapeutic compositions of the presently disclosed subject matter.

In some embodiments, the presently disclosed subject matter provides methods for inducing a target peptide-specific memory T cell response (*e.g.*, T_{CM}) response in a patient by providing the patient with a composition comprising the target peptides disclosed herein. In some embodiments, the compositions are those disclosed herein and are provided in a dosing regimen disclosed herein.

In some embodiments, the presently disclosed subject matter relates to methods for determining a cancer disease prognosis. These methods involve providing a patient with target peptide compositions and determining the extent to which the patient is able to mount a target peptide specific T cell response. In some embodiments, the target peptide

composition contains target peptides selected in the same substantially the same manner that one would select target peptides for inclusion in a therapeutic composition. If a patient is able to mount a significant target peptide-specific T cell response, then the patient is likely to have a better prognosis than a patient with the similar disease and therapeutic regimen that is not able to mount a target peptide-specific T cell response. In some embodiments, the methods involve determining whether the target peptide specific T cell response is a T_{CM} response. In some embodiments, the presence of a target peptide-specific T cell response as a result of the presently disclosed diagnostic methods correlates with an at least or about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 250, 300, 400, 500, or more percent increase in progression free survival over standard of care.

EXAMPLES

The following Examples provide illustrative embodiments. In light of the present disclosure and the general level of skill in the art, those of skill will appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter.

EXAMPLE 1

Identification of MHC Class I-associated Phosphopeptides (MHC-I-pP) as Novel Tumor-specific Antigens for HCC

Several methods exist for identification of tumor antigens on the surface of cancer cells. In the past, most often a "reverse immunology" approach was used, in which the peptide sequences of the tumor antigens were predicted *in silico*. MHC presented peptides with low binding affinities or those carrying posttranslational modifications cannot be predicted with this approach.

Using an approach involving direct isolation of MHC-peptide-complexes from the surface of the tumor cells, which is particularly useful for identification of post-translationally modified peptides, MHC class I-bound phosphopeptides (MHC-I-pP) were identified using the following general approach. Briefly, HCC tumors were removed and lysates were prepared from tumor tissue and adjacent (distal; normal) tissue. MHC-I-pP-complexes were immunoprecipitated from the HCC and adjacent (distal; normal) liver tissue lysates and affinity purified with the help of a MHC class I-specific antibody (W6/32; *see* Brodsky *et al.*, 1979). MHC-I-pP were separated and enriched from other MHC-bound peptides in several steps including elution and purification with a 10 kDa cut-

off filter and IMAC chromatography before the MHC-I-pP were characterized and sequenced by HPLC-ESI-MS/MS in a high-resolution mass spectrometer as described in Abelin *et al.*, 2015. Phosphopeptide sequences were manually assigned and comparisons were made between health and cancerous tissues.

As disclosed herein, 460 HCC-associated MHC-I-pP were identified. These data were acquired from four (4) different HCC samples and the corresponding adjacent cirrhotic or non-cirrhotic liver tissue and from a hepatoblastoma cell line (HepG2). In total, 21 HCC samples with the corresponding adjacent liver tissue were processed. Sequence data were derived from mass spectrometry analysis. Table 16 summarizes patient characteristics from the examined cohort.

Table 16

Patient Characteristics of the Cohort used for MHC-I-pP Identification on HCC Tumors and Adjacent Liver Tissue*

ID#	Age	HLA	Aetiology	C	CTP	BCLC	Treatment Received	AFP [kU/L]
SAMPLES FROM FEMALES								
3081	67	A*03 B*07 B*35	ALD	1	B	A	RFA 09.05.2011 and 24.04.2013	16
4164	39	A*02 A*03 B*15	Adenoma → HCC	0		A	Left hemihepatectomy	195
4233	74	A*01 A*02 B*08 C*07	DD ALD	0		A	Left hemihepatectomy	2414
4857	77	A*02 A*03 B*07 B*44 C*05 C*07	Adenoma → HCC	0		A	Resection	35850

4922	53	A*03 A*24 B*07 B*53 C*07 C*14	HBV	1	B	A	OLTx	6
5176	52	A*01 A*24 B*08 B*44 C*05 C*07	FNH -> HCC	0		A	Left lateral resection	-
5549	64	A*24 A*29 B*15 B*44 C*03 C*16	cryptogenic	0		A	Extended right hemihepatectomy	-
SAMPLES FROM MALES								
370	45	A*02 B*08 B*18 C*07	cryptogenic (Fibrolamellar HCC)	0		0	Resection	neg
981	81	A*01 A*02 B*27 B*37 C*02 C*06	?	1	A	0	Resection + RFTA in 02/10. Relapse -> PEI 11/11, 12/11, 01/11. Metastasis spine -> surgery 10/12	1
1515	60	A*02 A*03 B*18 C*05	ALD	1	A	A	OLTx	14

3907	53	A*02 A*26 B*08 B*49 C*07 C*07	DD ASH	1	B	0	Right hemihepatectomy	219
4028	48	A2+ B7-	HCV	1	A	0	OLTx	2
4908	80	A*01 A*24 B*08 B*15 C*03 C*07	A_1ATD	0		A	Resection	124
5437	58	A*02 A*03 B*15 B*40 C*03 C*04	HBV	1	A	A	OLTx	3
5487	79	A*03	HH	0		A	Caudate lobe resection	1
5493	65	A*03	NASH DD ALD	1	A	A	TACEx3 in 02/12, 07/12, 10/12. OLTx in 01/15.	1
TIL 12	64	A*02 A*30 B*18 B*35 C*04 C*05	ALD	1	B	A	OLTx	9
5573	54	A*01 A*03	HCV	1	B	A-B	OLTx	45
5721	57	B*08	HCV	1	A	A	Resection	-
5725	58	A*03	ALD	1	A	A	OLTx	16

* C: liver cirrhosis; CTP: Child-Turcotte-Pugh stadium; BCLC: Barcelona Clinic Liver Cancer Staging; AFP: α-fetoprotein; OLTx: Orthotopic liver transplantation.

Greater varieties of MHC-I-pP and on an average more MHC-I-pP were presented on tumor tissue than on premalignant liver cirrhosis or on non-cirrhotic liver tissue. Approximately 40 different MHC-I-pP were found per gram of tumor tissue and only around 10 MHC-I-pP were found per gram of non-cirrhotic liver tissue (*see* Figure 1A). The presentation of each MHC-I-pP per cell varied widely from statistically <1 copy/cell for most of the peptides up to 83 copies/cell. No differences with progression of the liver disease were observed (*see* Figure 1B). This might have been due to the fact that after the development of a cancer many bystander-mutations accumulate in the cancerous cells, which can lead to the presentation of a plethora of different MHC-I-pP on each cell.

29 out of the first 250 MHC-I-pP identified were discovered on healthy tissue, but most of them were found additionally on HCC tissue (n = 213), cirrhotic liver tissue (n = 19), and/or an HepG2 cells (n = 37). Most of the underlying proteins have not been previously associated with HCC. Some of the identified MHC-I-pP were found on other malignancies, *e.g.*, colorectal cancer (n = 109), esophageal cancers (n = 25; *see e.g.*, Tables 14 and 29), melanoma (n = 29), ovarian cancer (n = 38), hematological malignancies including leukemia (n = 75; *see also* Cobbold *et al.*, 2013), and breast cancer (n = 48), further highlighting the importance of this novel class of tumor antigens for cancer growths.

Overall, peptides restricted by several different MHC class I alleles have been identified. MHC-I-pP were predicted to bind most commonly to HLA-B*0702, HLA-B*2705, HLA-A*0201, and HLA-C*07. These data were potentially biased as 5 out of 5 of the analyzed samples were HLA-A*0201 positive, 3 out of 5 samples were HLA-A*C07 positive, but only one patient was HLA-B*0702 positive (*see* Figure 1C). Using a vaccination containing ~30 MHC-I-pP, it is possible that over ninety percent of the Caucasian population would be expected to recognize on average about 3 different MHC-I-pP (*see* Bui *et al.*, 2006).

The characteristics of HCC-specific HLA-A*0201-bound phosphopeptides was also investigated, which were similar to those previously reported for HLA-A*0201-bound phosphopeptides (Mohammed *et al.*, 2008). Briefly, each of the phosphopeptides was 7-13 amino acids in lengths and of 77 HLA-A*0201-restricted phosphopeptides, 70 contained a phosphoserine, 6 of the 77 contained a phosphothreonine, and 1 of the 77 contained a phosphotyrosine (*see* Table 2 and Figure 6). The phosphate was found at position 4 in 73% of HLA-A*0201 phosphopeptides (*see* Table 2 and Figure 6).

It has been reported that binding affinities of phosphopeptides are in general significantly greater than those of their non-phosphorylated counterparts and that this effect is most pronounced if the peptides are phosphorylated at P4 (*see* Mohammed *et al.*, 2008). Additionally, 55% of the phosphopeptides contained a positively charged amino acid (Arg or Lys) at P1, which seems to enhance the stability of the phosphopeptide-MHC association. HLA-A*0201-restricted phosphopeptides showed a strong preference for leucine at P2 and leucine/valine at P9 corresponding to the HLA-A*0201-supertype binding motif with a hydrophobic, aliphatic amino acid [L, I, V, M, A, T, Q] at position 2 and the C-terminal end (Sette & Sidney, 1999; Sidney *et al.*, 2008). Taken together, most HLA-A*0201-restricted phosphopeptides shared a common structure with a positively charged amino acid at position 1, a strong preference for leucine/valine at positions 2 and 9, and the phosphate moiety at position 4, which was oriented upwards, solvent oriented, and available for direct contact with the TCR (Mohammed *et al.*, 2008; *see* Figure 6).

EXAMPLE 2

Characterization of Immune Responses Against MHC-I-pP

Previous data had indicated that T cell responses against phosphoproteins can be found in healthy individuals and to a lesser extent in patients with malignant diseases (*see e.g.*, U.S. Patent Application Publication No. 2005/0277161; PCT International Patent Application Publication No. WO 2011/149909). These results suggested that individuals with a functional immune system create T cell responses against aberrantly phosphorylated peptides in order to eliminate those cells with signs of transformation. This may prevent further alterations and malignant transformation of the cells. A major goal of this project was to investigate if patients with chronic liver disease, HCC, and/or esophageal cancer are able to mount an efficient anti-phosphopeptide immune response during the course of disease.

From this, $CD8^+$ T cell responses against newly identified MHC class I-associated phosphopeptides in healthy individuals and patients with chronic liver disease were investigated. Twenty-one of the newly identified HCC-associated HLA-A*0201-restricted phosphopeptides were selected (*see* Table 17) for further immunological testing in HLA-A*0201 positive patients. MHC-I-pP-specific cytotoxic $CD8^+$ T cell responses (ppCTL) were assessed using intracellular cytokine staining (ICS) and several cytokines and surface markers were assessed in parallel. After 7 days of stimulation with the respective MHC-I-pP and no other cytokines, CD3- and CD8-expressing T cells were stained for at least two

different cytokines (IFN-γ, TNF-α) and when required CD107a expression as a marker for their cytotoxic potential.

First, peripheral blood mononuclear cells (PBMCs) from healthy donors and patients with hereditary hemochromatosis (HH) were analyzed. HH is a chronic liver disease characterized by excessive intestinal absorption of dietary iron resulting in a pathological deposition of iron in the liver. PBMCs or lymphocytes from liver tissue were extracted and specifically stimulated with (phospho-) peptides for 7 days before intracellular cytokine staining (ICS). Doublets and dead cells, using a fixable viability dye, were excluded. Lymphocytes were gated on $CD3^+$ and $CD8^+$ double positive cells and were analysed for expression of IFNγ, TNFα-, and CD107a.

Phosphopeptide-specific T cell responses were not found in healthy and young donors (HD) with a mean age of 26 years, although ppCTL-responses have been identified in healthy individuals – especially in middle-aged persons – by the instant co-inventors previously. Interestingly, ppCTL-responses were found in the peripheral blood of patients with chronic liver disease in the HH cohort in around 65% of cases (*see* Table 18). The patients in that cohort were significantly older with a mean age of 57 years. All of the HH patients were treated with phlebotomy and therefore liver disease was well controlled. None of the patients had abnormal liver function tests or abnormal ferritin values at the time of venesection (*see* Table 19). There was no correlation of immune responses against MHC-I-pP with the grade of liver injury, *e.g.*, steatosis, fibrosis, or cirrhosis.

ppCTL-responses were compared with responses to immunodominant viral epitopes from cytomegalovirus (NLVPMVATV; SEQ ID NO: 451) and Epstein-Barr virus (GLCTLVAML; SEQ ID NO: 501). In most cases, T cell responses against MHC-I-pP were comparable in quantity and quality to viral immune responses (*see* Figures 2 and 3A; *see also* Table 18). This is in contrast to the "classic" TAA, where immune responses are often nearly not detectable (< 0.1 % of $CD8^+$ T cells) and often show signs of exhaustion (Flecken *et al.*, 2014). In the instant analysis, only responses with a minimum of 0.25% of reactive $CD8^+$ T cells were considered positive. ppCTLs produce multiple cytokines, mainly IFNγ and TNFα (*see* Figure 3A), but also low amounts of IL-2. The production of multiple cytokines (IFNγ, TNFα and IL-2) by T lymphocytes, including the capacity to degranulate (measured by the surface expression of CD107a) is in general associated with better disease control (Almeida *et al.*, 2007; Harari *et al.*, 2007).

Approximately one-third of the ppCTLs were positive for the degranulation marker CD107a, indicating their ability to kill cancer cells. There was a slight tendency of

ppCTLs to produce larger amounts of TNFα in comparison to virus-specific $CD8^+$ T cells, which did not turn out to be significant. This suggested that TNFα was a more sensitive marker for detecting ppCTLs than IFNγ or CD107a.

ppCTLs are mainly $CD27^+$ and $CD45RA^-$ and therefore most-likely reside in the memory compartment (*see* Figure 3B). This suggested that only individuals that had been previously exposed to the MHC-I-pP established an immunological memory against these antigens. If healthy donors were too young, like in the instantly described healthy control group (mean age ~26 years), they likely did not yet have the chance to be exposed to MHC-I-pP tumor antigens. However, if patients had an underlying chronic disease which predisposed them to the development of a cancer, such as like in the instant HH cohort, then phosphopeptide immune responses were measurable in over 60% of cases.

Exhausted TAA-specific T cells in the cancer microenvironment express high levels of inhibitory receptors, including PD-1 and CTLA-4, and show impaired effector cytokine/molecule production, such as IL-2, TNF-α, IFN-γ, and CD107a. PD-1- and CTLA-4 expression was measured on the surface of ppCTLs-derived from PBMCs of patients with chronic liver disease. ppCTLs expressed more CTLA-4 on their surface than virus-specific T cells from the same patients (*see* Figures 2, 7, and 8). PD-1 expression did not seem to be increased on the surface of ppCTLs. PD-1 expression is usually upregulated on tumor-infiltrating $CD8^+$ T cells and correlates with reduced cytokine production in hepatocellular carcinoma (Bui *et al.*, 2006) and other cancer patients. $PD\text{-}1^+$ and $CTLA\text{-}4^+$ double positive $CD8^+$ TILs are even more severely exhausted in proliferation and cytokine production and dual blockade with monoclonal antibodies enhances T cell function in cancer (Takayama *et al.*, 2000). The mixed pattern described herein suggested that ppCTLs were in an intermediate stage and not yet fully exhausted, at least in the peripheral blood. This favored a CTLA-4 monoclonal antibody therapy for restoring immunity against phosphopeptide tumor antigens in patients with chronic liver disease.

Specific ppCTL-lines were enriched from PBMCs with multiple rounds of stimulation against the respective phosphopeptides. A ppCTL-line against the protein serine/arginine-rich splicing factor 8 (SRSF8) secreted IFNγ, TNFα and expressed CD107a in response to stimulation only with the phosphorylated peptide IMDRtPEKL (SEQ ID NO: 14), but not to stimulation with unphosphorylated IMDRTPEKL (SEQ ID NO: 14) peptide, suggesting that recognition of MHC-I-pP in patients with chronic liver disease could be exclusively phosphate-dependent. In one HH patient, a response against

the MHC-I-pP RVAsPTSGV (SEQ ID NO: 57) from the protein insulin receptor substrate 2 (Irs2) was even evident *ex vivo* in an ICS from PBMCs of a patient with hereditary hemochromatosis. The observation that it was possible to detect *ex vivo* T cell responses against MHC-I-pP was important because *in vitro* stimulation resulted in quantitative and functional changes of T cell responses.

EXAMPLE 3

Initiation and Expansion of Phosphopeptide-specific $CD8^+$ T Cells for Adoptive T Cell Transfer (ACT) Therapy

It has been shown that adoptive cell transfer (ACT) of TILs can mediate cancer regression in patients with metastatic melanoma (Rosenberg & Restifo, 2015). In ACT, autologous immune cells from a patient are removed, altered and/or expanded *in vitro*, and then transferred back into the patient in order to kill cancer cells. It is still unclear, however, whether this approach can be applied to primary liver cancer or for targeting phosphopeptide tumor antigens.

It is a widely accepted hypothesis that a greater concentration of tumor-reactive lymphocytes can be found at tumor sites in comparison to the peripheral blood. Therefore, whether anti-phosphopeptide immune responses could be found in tumor-infiltrating lymphocytes (TILs) from HCC or in the liver compartment in general was investigated. Different protocols for intrahepatic lymphocyte (IHL) and tumor-infiltrating lymphocyte (TIL) isolation and purification exist (Morsy *et al.*, 2005). Resected tissue specimen are either digested into a single-cell suspension (enzymatic digestion, ED) or divided into multiple tumor fragments that are individually grown in IL-2 (Dudley *et al.*, 2003). It was a goal to understand which technique works best for liver tissue and from which compartment ppCTLs had to be extracted in order to expand ppCTLs for ACT.

In addition, several methods for expanding tumor reactive TILs have been described. Late successes in clinical trials using ACT for melanoma and epithelial cancers ACT used a technique for expanding TILs called rapid expansion protocol (REP) described in Dudley *et al.*, 2003. With this technique, cultures are rapidly expanded in the presence of excess irradiated feeder lymphocytes, anti-CD3-antibody, and high-dose IL-2. So far, it is unclear if expansion of ppCTLs with REP has been successful for liver-derived lymphocytes and ppCTLs.

To test the feasibility of ACT with ppCTLs for patients with advanced HCC, different published extraction protocols described in Morsy *et al.*, 2005 were tested and

the proliferative potential, phenotype, and antigen specificity of expanded liver-derived ppCTLs were assessed.

A total of 41 liver specimens from explanted livers after orthotopic liver transplantation (OLTx) or from resection or from deceased donor livers (DDL) that were rejected for transplantation. In total, specimens were obtained from 6 DDLs, 5 from end-stage liver cirrhosis, and 17 from HCC patients. In each case attempts were made to obtain both tumor and adjacent tissue. Clinical parameters of the patients are summarized in Table 20. Most of the specimens came from explanted organs after transplantation and consequently most livers were severely cirrhotic.

Initiation of TIL microcultures from tissue fragments (TF) and by enzymatic digestion (ED) from tumor samples were compared. 14 out of 17 HCC tumors were minced into fragments and 10 out of 17 samples were processed into single cell suspensions by ED. 6 smaller tumors were only minced into fragments (Table 21). Initiation of lymphocyte cultures worked both for TF and ED with tumor tissue, but for adjacent tissue (distal liver tissue, 2 cm or more away from the tumor), ED was the preferred method. Initiation of microcultures from TF from HCC led to viable cell numbers in around sixty percent of cases. This is in accordance with published results from generation of TILs from gastrointestinal-tract cancer liver metastases (Turcotte *et al.*, 2013). T cell cultures initiated by TF from liver specimens distal to the tumor often failed to induce viable T cell cultures. In contrast, initiation of cultures by ED was possible in 70-80% of cases for both tumor and distal tissue.

Lymphocyte populations from TF reached a confluent lymphocytic carpet, which was countable, after ~14 days of culture. Until that time, cultures derived by ED had already nearly doubled. Growth of lymphocytes derived by ED in most cases outperformed cultures initiated from TF in the first 2-4 weeks.

To further characterize the cultures, cultures were analyzed by flow cytometry including multiple markers (CD3, CD4, CD8, CCR7, CD45RO, CD25, FoxP3) between weeks 5-7. Interestingly, significant differences were observed in the composition of the cultures derived by TF or ED. Cultures derived by ED yielded higher number of $CD8^+$ T cells in comparison to cultures initiated with TF. In cultures from TF, $CD4^+$ T cells were the predominant population. No major differences were observed in terms of CD8 T cell marker expression or CD4 markers (Table 22) and were comparable to results published for other cancers (Turcotte *et al.*, 2014).

These results suggested that obtaining lymphocytes from TF, which was extensively used in the past for ACT in malignant melanoma and other cancers, did not seem to be the optimal method for patients with HCC. In >90 of cases, the tissue adjacent to the HCC was severely cirrhotic and this seemed to prevent exit of lymphocytes out of the tissue into the culture. Therefore, approaches in which help is given to the lymphocytes by mechanical and enzymatic disaggregation of the cirrhotic tissue seem to be preferable.

A problem that arises from ED is that larger tissue samples are needed in order to get a sufficient number of lymphocytes to start a culture. That would mean that patients with HCC would need to have surgery before ACT in order to acquire enough tumor tissue. But that is not practical considering the expected symptoms from liver cirrhosis, which would be expected to be exacerbated by surgery. A possible approach to obtain liver tissue before immunotherapy could thus be liver biopsy.

After initial outgrowth of the hepatic lymphocyte cultures, whether TILs or IHLs could be expanded in large quantities using a standard 14-day rapid expansion protocol (REP) with irradiated PBMC feeders, soluble anti-CD3 antibody, and high-dose IL-2 was tested. For all the cultures tested, an expansion of the T cells up to 1 x 10^9 cells was achieved within the first 14-21 days. No differences were observed in the potential to expand lymphocytes derived from healthy liver tissue, cirrhotic liver tissue, or HCCs (*see* Figure 4A). A further expansion was also possible but not investigated.

Positive selection of $CD8^+$ TILs prior to REP was performed with magnetic beads in seven of the samples. Growth was accelerated in the first 14 days with $CD8^+$ pre-selected T cell cultures (*see* Figure 4B). It has previously been reported that a clinical grade expansion of TILs in melanoma and GI tract cancers was identical for unselected and CD8 pre-selected cultures (Prieto *et al.*, 2010; Turcotte *et al.*, 2014). Again, expanded cultures were further classified and phenotyped, and no major differences of the examined markers were observed pre- or post-expansion.

Taken together, expansion of liver-derived lymphocytes was easily accomplished with the REP protocol and was not dependent on the origin of the lymphocytes. Next, the expanded lymphocyte cultures were screen for MHC-I-pP reactivity. The expanded T cell cultures were stimulated with the respective phosphopeptides and analyzed 7 days later with ICS in the same way as described herein above for PBMCs.

Interestingly, only very few and minor responses were detectable in all of the cultures. Background cytokine production was much higher in expanded lymphocyte

cultures and therefore often genuine T cell responses were difficult to distinguish from background. Responses, which were demonstrated in the unexpanded cultures, were completely absent in the expanded T cell cultures (*see* Table 23 and Figure 5A). Interestingly virus-specific T cell reactivity was not lost during expansion of T cells. A Box and Whiskers plot (*see* Figure 5B) of the data from Table 23 calculated with Graph Pad showed that ppCTLs after expansion were functional, produced multiple cytokines, and were able to degranulate.

This indicated that if expansion of T cells happened in a large scale and in an undirected way, virus-specific T cells and tumor-unspecific T cells overgrew tumor-specific T cells. This might be one reason why ACT with T cells failed to induce clinical responses on a regular basis. Overgrowth of virus-specific cells could be due to the fact that these cells were less exhausted and expressed lower amounts of inhibiting receptors (*see* Figure 3C).

Therefore, lymphocyte cultures were repeatedly stimulated before and during the expansion reaction with a phosphopeptide-pool (*see* Table 3). With this phosphopeptide-specific expansion, lost immune responses against phosphopeptides could be restored and could be clearly identified from the background (*ssee* Table 23 and Figure 5A) in most cases. Depending on the reaction, not every clone was expanded every time, but the strongest immune responses were conserved before and after expansion. Again ppCTLs after expansion were able to produce multiple cytokines and the degranulation marker CD107a, indicating that expanded cells are fully functional after REP for ACT.

Finally, if ppCTLs could be found in TILs or the liver compartment in general was investigated. Because T cell numbers were small after initiation of the cultures, it was necessary to look in expanded T cell cultures. Therefore, all available HLA-A*0201 positive cultures derived by ED from our 3 cohorts were expanded with our new phosphopeptide-specific expansion protocol. The expanded T cells were individually stimulated with the 21 HLA-A*0201 restricted MHC-I-pP (*see* Table 3) for another 7 days before ICS.

Interestingly, most of the responses against MHC-I-pP were found in the cultures derived from "healthy" deceased donor livers. Only a few responses could be found in cultures derived from end-stage liver cirrhosis, although one of the responses was very strong and consisted of greater than 15% of the whole $CD8^+$ T cell population. In the HCC livers, no ppCTLs could be found or expanded, neither in the tumor itself nor in the adjacent tissue. These results were consistent with observations reported for leukemia-

associated phosphopeptides (Cobbold *et al.*, 2013), where only in very few patients with cancer ppCTLs could be found.

These results suggested that tumor outgrowth was accompanied by immunosuppressive mechanisms in the tumor microenvironment and T cell exhaustion, which led to the disappearance of anti-phosphopeptide immunity during the course of disease.

Table 24 provides a summary of all ppCTL responses from pP-specifically expanded cultures from "healthy" livers, cirrhotic livers. and HCCs.

Discussion of the EXAMPLES

HCC develops normally after several years of chronic liver inflammation and most of the time after the development of liver cirrhosis. In the course of chronic liver diseases several mutations and epigenetic changes accumulate in the liver cells which finally lead to a dysregulation of major signaling pathways that are important for malignant transformation (Whittaker *et al.*, 2010). Other current studies suggest that HCC can be derived from cancer stem cells (CSCs) in preneoplastic regions of altered hepatocytes (He *et al.*, 2013). Taken together, HCC is considered to be a slowly developing malignancy to evolve from premalignant lesions in chronically damaged livers.

Therefore, it was hypothesized that phosphopeptides are presented increasingly on the surface of altered hepatocytes with progression of the disease. Young and healthy individuals are likely to clear altered, premalignant hepatocytes with the help of phosphopeptide-specific cytotoxic lymphocytes. As liver disease progresses and liver damage increases the immune system is not able to clear all the cancer progenitors and defensive mechanisms of the early tumors against the immune system gain the upper hand. Therefore, a loss of immune responses against phosphopeptides during disease progression could be a predictor for poor outcomes in patients with HCC.

Disclosed herein are 460 phosphopeptides presented to the immune system by MHC molecules derived from human hepatocellular carcinoma and/or esophageal carcinoma. It is noted that there are hundreds of different HLA alleles in the human population, and each individual expresses 3 to 6 different alleles. With respect to Caucasians, for example, most carry at least one of the following six alleles: HLA A*0201 (50%); HLA A*0101 (29%); HLA A*0301 (21%); HLA B*4402 (27%); HLA B*0702 (30%); and HLA B*2705 (7%). Since disclosed herein are phosphopeptides presented by all of these HLA alleles, it should be possible to treat heptatocellular carcinoma in

approximately 90% percent of the Caucasian population using the compositions comprising the phosphopeptides disclosed herein.

Many of the underlying proteins from the identified respective MHC-I-pP can be directly linked to important HCC-characteristic, malignant signaling pathways (Whittaker *et al.*, 2010), which highlight their importance as potential new immunotherapeutic targets. Functional annotation clustering of all identified MHC-I-pP with respect to their biological processes (GO term BP analysis) using the Database for Annotation, Visualization and Integrated Discovery v6.7 (DAVID; Huang da *et al.*, 2009) yielded several enriched clusters of proteins involved in transcriptional regulation, cell cycle regulation, regulation of metabolic processes, apoptosis, cell death, cell migration, and many other biological processes, which have been associated with "hallmarks of cancer" (Hanahan & Weinberg, 2011; *see also* Table 25). Biocarta and KEGG signaling pathway mapping of all identified MHC-I-pP revealed that HCC-specific MHC-I-pP are significantly enriched in mitogen-activated protein kinase (MAPK) pathways and the Neurotrophin pathway (see Table 26). Several studies also indicate a major role of the MAPK/RAF/MEK/ERK pathways in the tumorigenesis of HCC3. This is in contrast to the "classical" TAA and cancer-associated HLA-lingandome, for which cluster formation is not observed and associations to biological processes or overrepresented pathways cannot be found (Kowalewski *et al.*, 2015). However, due to the incomplete data set, small sample size, and low enrichment scores of these clusters, the data does not represent a complete picture of the involvement of phosphopeptides into important biological functions and their pathways yet.

Further highlighting the central position in major cancer associated pathways is the fact that some of the underlying proteins are covered by several MHC-I-pP. Those were found on different HCC and/or esophageal cancer samples and were presented by different HLA molecules. This might indicate that key proteins for malignant transformation are presented as phosphopeptides by the immune system across "HLA-borders". For example, two MHC-I-pPs, KRYsGNmEY (SEQ ID NO: 242) and RRDsLQKPGL (SEQ ID NO: 248), were identified for the serine/threonine protein kinases LATS1 and LATS2 (*see* Table 27), predicted to bind to HLA-C*07 and HLA-B*2705. LATS1 and LATS2 have been shown to be negative regulators of YAP1 in the Hippo signalling pathway (Hao *et al.*, 2008). Two different MHC-I-pP were identified for the Mitogen-activated protein kinase kinase kinase 3 and 11 (MAP3K3/11), which play a key role in the MAPK/ERK/JUN-signalling cascade and activation of B-Raf, ERK and cell proliferation (Tibbles *et al.*, 1996; Ellinger-Ziegelbauer *et al.*, 1997; Chadee & Kyriakis, 2004). Both

peptides are predicted to bind to different MHC molecules, HLA-B*2705 and HLA-B*0702, respectively, and additionally were found on different cancers too.

Several of the phosphopeptides were identified on more than one type of cancer. These are summarized in Table 28.

While not wishing to be bound by any particular theory of operation, chronic liver diseases such as chronic hepatitis B or C infection (HBV/HCV), alcohol, non-alcoholic steatohepatitis (NASH), or autoimmunehepatitis (AIH) can lead to chronic inflammation of the liver with subsequent multiple changes in signaling pathways, oncogenes, and tumor suppressor genes (*see e.g.*, Whittaker *et al.*, 2010). Most of these processes are mediated with the help of kinases and phosphorylation of signaling pathways. HCC-specific phosphopeptides appear to be presented with increasing amounts on the surface of altered hepatocytes during disease progression. This can leads to an immune response against the hepatocytes showing signs of malignant transformation. During progression of liver disease towards HCC, immunosuppressive mechanisms can gain the upper hand and phosphopeptide-specific immunity can be lost.

Taken together, it was observed that phosphopeptide neoantigens could be identified on human primary liver cancers and/or esophageal cancers that were immunogenic in certain cohorts of patients. In total, 460 MHC class-I restricted phosphopeptides were identified, and it was demonstrated that more antigens were presented during the course of chronic liver disease towards development of HCC. Many of the HCC-specific MHC-I-pP were derived from genes directly linked to important functions for tumorigenesis, making these particularly interesting as immunotherapeutic targets. MHC-I-pP seemed to be the target of a pre-existing immunity, ppCTLs were functional and most likely were able to kill cancer cells. Interestingly, it seemed that patients with chronic liver disease did lose the ability to destroy cancer cells with the help of ppCTLs during the course of the disease towards end-stage liver disease. Thus, enhancing immunity against these tumor-associated antigens should provide a cancer immunotherapeutic strategy.

Adoptive T cell transfer therapy for HCC has been performed in very few clinical trials to date (Rosenberg *et al.*, 1985; Takayama *et al.*, 2000; Hui *et al.*, 2009; Shimizu *et al.*, 2014), and in all of these trials cells have been expanded using different methodologies. Disclosed herein is demonstrated that it was possible to grow and expand ppCTLs in a large scale for ACT using a directed and improved rapid expansion protocol.

It is further disclosed herein that these cells remained functional and specific after expansion.

The results of the presently disclosed experiments implied that the main challenge to develop an effective adoptive T cell therapy for HCC and/or esophageal cancer using patient-derived lymphocytes might not be the *in vitro* proliferative capacity of the lymphocytes, but rather the selection and enrichment of tumor-reactive and in particular of phosphopeptide-specific T cells.

Furthermore, survival of these tumor-reactive T cells over a long period of time during expansion should be guaranteed in order to treat patients with "useful" anti-cancer lymphocytes. Lately, the poor therapeutic efficacy of autologous T cells against the tumor antigens gp100 and MART-1 raised significant concerns targeting this class of tumor antigens (Chandran *et al.*, 2015). This and the fact that phosphopeptides are especially immunogenic make this class of tumor-associated antigens particularly interesting for use in immunotherapy.

REFERENCES

All references listed in the instant disclosure, including but not limited to all patents, patent applications and publications thereof, scientific journal articles, and database entries (including but not limited to UniProt, EMBL, and GENBANK® biosequence database entries and including all annotations available therein) are incorporated herein by reference in their entireties to the extent that they supplement, explain, provide a background for, and/or teach methodology, techniques, and/or compositions employed herein. The discussion of the references is intended merely to summarize the assertions made by their authors. No admission is made that any reference (or a portion of any reference) is relevant prior art. Applicants reserve the right to challenge the accuracy and pertinence of any cited reference.

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It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

Table 17

Exemplary HCC-associated HLA-A*0201-restricted MHC-I-pP Selected for Further Immunological Testing

MHC-I-pP	Copies/ cell	Found on other cancers	Function / Significance in cancer
ALDsGASLLHL (SEQ ID NO: 2)	0.1 – 0.4	Ovarian Ca, Hematological Ca, Breast Ca, CRC	Involved in stratified epithelial development. It is a direct transcriptional target of TP63. Plays a role in NF-kappa-B activation.
AVVsPPALHNA (SEQ ID NO: 6)	0.5 – 0.7	Ovarian Ca, Hematological Ca.	Chromatin reader protein that plays a key role in transmission of epigenetic memory across cell divisions and transcription regulation. Maintains high-order chromatin structure. Also acts as a regulator of p53/TP53-mediated transcription following phosphorylation by CK2.
FLDtPIAKV (SEQ ID NO: 9)	0.1 – 0.15	CRC.	Antagonist of Wnt-signaling.
IMDRtPEKL (SEQ ID NO: 14)	0.2	Ovarian Ca, CRC, OEAC, Hematological Ca, Melanoma, Breast Ca	Adapter protein that couples growth factor receptors to a signaling pathways that regulate the proliferation in breast cancer cells. When overexpressed, it confers anti-estrogen resistance in breast cancer cell lines.
KAFsPVRSV (SEQ ID NO: 16)	0.1 – 81	Ovarian Ca, CRC, OEAC	Transcriptional regulator (lacking a basic DNA binding domain) including cellular growth, senescence, differentiation, apoptosis, angiogenesis, and neoplastic transformation.
KIAsEIAQL (SEQ ID NO: 17)	0.3 – 0.8	Ovarian Ca	Unknown.
KLFPDtPLAL (SEQ ID NO: 21)	0.1 – 0.3	Ovarian Ca, CRC, Melanoma, Hematological Ca	May facilitate double-stranded RNA-regulated gene expression at the level of post-transcription. Can act as a translation inhibitory protein.

RLAsYLDRV (SEQ ID NO: 34)	0.4 – 1.8	CRC	Filament reorganization. Migration and invasion of tumor cells.
RLFsKELRC (SEQ ID NO: 39)	0.3	Ovarian Ca, Melanoma	Transcription initiation factor of RNA Pol II.
RLSsPLHFV (SEQ ID NO: 43)	0.3 -1	CRC, Melanoma, Hematological Ca	Unknown.
RQAsIELPSMAV (SEQ ID NO: 46)	0.1	CRC, Hematological Ca	May play a role in mediating neutrophil activation and chemotaxis. Significance in cancer unclear.
RQDsTPGKVFL (SEQ ID NO: 48)	1.1 – 8.4	Ovarian Ca, CRC, Melanoma, Hematological Ca.	Orphan nuclear receptor. Primarily repressor of a broad range of genes. Binds to hormone response elements (HREs). Together with NR2C2, forms the core of the DRED complex that represses embryonic and fetal globin transcription. May be involved in stem cell proliferation and differentiation.
RQLsSGVSEI (SEQ ID NO: 51)	0.9	Ovarian Ca, Melanoma, Hematological Ca, CRC	Involved in stress resistance and actin organization.
RTFsPTYGL (SEQ ID NO: 54)	0.3 – 6.8	Ovarian Ca, Melanoma, OEAC	Type-VI intermediate filament (IF) which plays an important cytoskeletal role within the muscle cell cytoskeleton.
RVAsPTSGV (SEQ ID NO: 57)	0.4 – 0.8	Ovarian Ca, CRC, Melanoma	Mediates the control of various cellular processes by insulin e.g. GH-, PI3K/AKT-, IGF1R-, Leptin-signaling.
SImsPEIQL (SEQ ID NO: 58)	0.25	Ovarian Ca	Transcriptional repressor which may play a role in development of the central nervous system (CNS).

SMTRsPPRV (SEQ ID NO: 70)	14	Ovarian Ca, CRC, OEAC, Hematological Ca, Melanoma	mRNA splicing and insulin synthesis.
VMIGsPKKV (SEQ ID NO: 76)	2-3	Ovarian Ca, CRC, OEAC, Hematological Ca, Melanoma.	Cell migration and invasion.
yLQSRYYRA (SEQ ID NO: 77)	0.2 – 1.9	Ovarian Ca	Kinase involved in transcription regulation, apoptosis and steroidogenic gene expression. Phosphorylates JUN and RUNX2.
PmVTLsLNL (415)	6	NA	Unknown. Interacts with p53 and NEDD1 and VCAM.
RTHsLLLLL (SEQ ID NO: 428)	1-32	Ovarian Ca, CRC, OEAC, Hematological Ca, Melanoma	Unknown. Is secreted by colorectal cancer cell line.

Table 18

Summary of Reactive $CD8^+$ T Cell Populations to HLA-A*02-specific Phosphopeptides

	Healthy Donors (mean age ~ 26 years)					HH Patients (mean age ~57 years)					
Sequence	HD1	HD2	HD3	HD4	HD5	HH2	HH3	HH6	HH8	HH10	HH11
NLVPMVATV (SEQ ID NO: 451)	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**1**	**1**	**3**	**0**
GLCTLVAML (SEQ ID NO: 501)	**2**	**0**	**0**	**2**	**1**	**3**	**0**	**0**	**0**	**6**	**2**

RTHsLLLLL (SEQ ID NO: 428)	0	0	0	0	0	0	0	0	0	0	0	0
SMTRsPPRV (SEQ ID NO: 70)	0	0	0	0	0	0	0	0	0	0	0	0
VMIGsPKKV (SEQ ID NO: 76)	0	0	0	0	0	0	0	0	0	0	1	0
IMDRtPEKL (SEQ ID NO: 14)	0	0	0	0	0	0	0	1	0	0	0	0
RVAsPTSGV (SEQ ID NO: 57)	0	0	0	0	0	0	0	0	0	0	2	0
RLAsYLDRV (SEQ ID NO: 34)	0	nd	nd	0	0	3	0	0	0	nd	nd	nd
yLQSRYYRA (SEQ ID NO: 77)	0	nd	nd	0	0	0	0	1	0	nd	nd	nd
PmVTLsLNL (SEQ ID NO: 415)	0	nd	nd	0	0	0	0	0	0	nd	nd	nd
KAFsPVRSV (SEQ ID NO: 16)	0	nd	nd	0	0	0	0	0	0	nd	nd	0
FLDtPIAKV (SEQ ID NO: 9)	0	nd	nd	0	0	0	0	0	0	nd	nd	0
KIAsEIAQL (SEQ ID NO: 17)	0	nd	nd	0	0	0	0	1	0	nd	nd	nd
RLSsPLHFV (SEQ ID NO: 43)	0	nd	nd	0	0	1	0	0	0	nd	nd	0
RTFsPTYGL (SEQ ID NO: 54)	0	nd	nd	0	0	0	0	0	0	nd	nd	0
SImsPEIQL (SEQ ID NO: 58)	0	nd	nd	0	0	1	0	0	0	nd	nd	nd
ALDsGASLLHL (SEQ ID NO: 2)	0	nd	nd	0	0	0	0	0	0	nd	nd	1
AVVsPPALHNA (SEQ ID NO: 6)	0	nd	nd	0	0	0	0	2	0	nd	nd	nd
KLFPDtPLAL (SEQ ID NO: 21)	nd	nd	nd	nd	nd	0	0	nd	0	nd	nd	0
RLFsKELRC (SEQ ID NO: 39)	nd	nd	nd	nd	nd	2	0	nd	0	nd	nd	nd
RQAsIELPSMAV (SEQ ID NO: 46)	nd	nd	nd	nd	nd	0	0	nd	0	nd	nd	0
RQDsTPGKVFL (SEQ ID NO: 48)	nd	nd	nd	nd	nd	0	0	nd	0	nd	nd	0

RQLsSGVSEI (SEQ ID NO: 51)	nd	nd	nd	nd	nd	0	0	nd	0	nd	0

Phospho-Ser, phospho-Thr, and phosphor-Tyr residues are indicated by s, t, and y, respectively. Oxidized methionine residues are depicted by "m".

nd: not determined; 0: <0.25% reactive $CD8^{+}$ T cells; 1: 0.25-2.5% reactive $CD8^{+}$ T cells; 2: 2.5-5.0% reactive $CD8^{+}$ T cells; 3: 5-7.5% reactive $CD8^{+}$ T cells; 4: 7.5-10% reactive $CD8^{+}$ T cells; 5: 10-20% reactive $CD8^{+}$ T cells; 6: >20% reactive $CD8^{+}$ T cells.

Table 19

Characteristics of the HH Patients

Characteristic	Hereditary Hemochromatosis	Healthy Population
Mean age (range) - yr	57 (40 – 70)	26 (22 – 34)
Male sex - %	73	20
Genetic background - %		
- C282Y homozygous	75	-
- C282Y/H63D compound	25	-
Steatosis - %	45	0
Fibrosis - %	27	0
Cirrhosis - %	0	0
HCC - %	0	0
Treatment	All received phlebotomy	No treatment

Lab values	± SEM	
WBC [g/L]	5.61 ± 3.37	4 - 11
Hb [g/L]	149.64 ± 3.09	135 - 180
Plts [*10^9/L]	223.64 ± 14.6	150 – 450
Creatinine [µmol/L]	82.73 ± 6.22	60 – 120
GPT (ALT) [U/L]	24.36 ± 2.46	10 – 50
AP [U/L]	65.55 ± 3.59	40 – 130
Bilirubin [µmol/L]	18.09 ± 4.27	< 22
INR	1.05 ± 0.03	0.85 – 1.15
Ferritin [µg/L] (range)	99.7 ± 21.54 (26 – 268)	18 - 360

Table 20

Characteristics of Patients from Whom Liver Tissue was Obtained for Lymphocyte Extraction, Expansion, and Testing for MHC-I-pP Immune Responses

Characteristic	Deceased donor livers (DDL)	Liver cirrhosis patients	HCC patients
Mean age (range) - yr	?	43.5 (34 – 53)	67.7 (52 – 82)
Male sex - %	?	60	82

Chronic liver disease - %			
ALD / NASH		-	29
HBV/HCV		-	35
AIH / PBC / PSC		60	0
Others		40	35
Fibrosis - %	0	20	18
Cirrhosis - %	0	80	65
Child-Turcotte-Pugh-stadium - %			
- A	-	40	55
- B	-	60	45
- C	-	0	0
BCLC - %			
- A	-	-	76
- B	-	-	24
- C	-	-	-
- D	-	-	-
Treatment - %			
- Resection / Surgery	-	0	41
- OLTx	-	100	59
Lab values ± SEM			
AFP [kU/L]		1.3 ± 0.4	2598 ± 4124

WBC [g/L]	6.6 ± 6.6	5.9 ± 0.9
Hb [g/L]	109.0 ± 3.1	135.6 ± 11.9
Plts [*10^9/L]	134.7 ± 29.3	159.2 ± 47.8
Creatinine [µmol/L]	47.0 ± 5.4	74.2 ± 14.1
GPT (ALT) [U/L]	83.0 ± 13.9	36.6 ± 8.1
AP [U/L]	783.3 ± 168.8	152.1 ± 29.2
Bilirubin [µmol/L]	184.0 ± 25.9	22.6 ± 8.8
INR	1.2 ± 0.1	1.3 ± 0.1

Table 21

Initiation of TIL Cultures by ED or TF

		Initiation by[a]				Successful[b]			
		TF		ED		TF		ED	
Sample ID		Tu	Di	Tu	Di	Tu	Di	Tu	Di
LL4857	TIL1	+	-	-	+	+			+
LL4908	TIL2	+	+	-	+	+	-		-
LL4922	TIL3	+	+	+	+	-	+	+	+
LL4959	TIL4	Ø	-	Ø	+				+
LL5176	TIL5	+	Ø	+	Ø	-		+	
LL5210	TIL6	+	+	+	+	+	-	-	+

LL5259	TIL8	Ø	-	Ø	+				+
LL5437	TIL9	+	+	-	+	+	-		+
LL5487	TIL10	+	Ø	+	Ø	+		+	
LL5493	TIL11	+	+	-	+	-	-		+
LLUNKN	TIL12	+	+	-	+	-	+		+
LL5549	TIL13	+	+	+	+	-	-	+	+
LL5573	TIL14	+	+	+	+	+	-	+	+
LL5721	TIL15	+	+	+	+	+	-	-	+
LL5725	TIL16	+	-	-	+	+			+
LL5737	TIL17	+	+	+	+	-	-	-	+
LL5975	TIL18	-	-	+	+			+	-

TF: tissue fragments; ED: enzymatic digestion; Tu: tumor tissue; Di: distal (normal tissue)

[a] in these columns, "+" represents that an attempt was made to establish a culture from the corresponding sample, "-" represents that an attempt was not made to establish a culture from the corresponding sample, and "Ø" represents that tissue was not available for this sample.

[b] in these columns, "+" represents that a culture was successfully established from the corresponding sample and "-" represents that a culture was not successfully established from the corresponding sample.

Table 22

Classification of TIL Cultures on the Basis of Surface Marker Expression Between Weeks 5 and 7

	naive *CCR7+ CD45RA+*	**EM** *CCR7- CD45RA-*	**EMRA** *CCD7- CD45RA+*	**CM** *CCR7+ CD45RA-*	**Tregs** *CD25+ FoxP3+*
TF Tu	6.5 ± 5.5	37.5 ± 14.0	47.3 ± 29.0	11.8 ± 6.8	1.6 ± 1.3
TF Di	10.6 ± 0.0	58.1 ± 0.0	20.0 ± 0.0	11.3 ± 0.0	0.9 ± 0.0
ED Tu	5.0 ± 1.3	53.8 ± 15.4	38.1 ± 15.8	3.1 ± 3.3	6.8 ± 5.7
ED Di	3.4 ± 3.1	69.5 ± 21.6	23.3 ± 23.8	3.8 ± 4.2	2.3 ± 1.0
DDLs	6.4 ± 5.2	71.1 ± 3.7	8.4 ± 3.4	14.1 ± 5.8	5.0 ± 3.7
IHLs	22.2 ± 12.8	27.1 ± 12.9	50.0 ± 0.6	0.7 ± 0.5	24.8 ± 32.8
REP	1.64 ± 1.68	55.76 ± 12.89	1.02 ± 1.50	41.58 ± 13.37	n.d.
PBMCs	33.8 ± 27.8	28.0 ± 14.2	24.1 ± 9.7	14.1 ± 6.3	1.0 ± 1.2

Data are presented as mean ± SD.

Abbreviations: EM: effector memory T cell; EMRA: effector memory T cell expressing CD45RA; CM: central memory T cell; Tregs: regulatory T cell.

Table 23

Effect of Antigen-specific Expansion on Tumor-infiltrating Lymphocyte Cultures

	No Expansion			Non-specific Expansion			pP-specific Expansion		
Sequence (SEQ ID NO:)	HH6	Do2	Do4	HH6	Do2	Do4	HH6	Do2	Do4
NLVPMVATV (451)	**1**	**nd**	**2**	**1**	**0**	**5**	**3**	**3**	**2**
GLCTLVAML (501)	**0**	**nd**	**1**	**0**	**2**	**3**	**4**	**3**	**3**
RTHsLLLLL (428)	**1**	**nd**	**2**	**1**	**0**	**0**	**0**	**0**	**0**
SMTRsPPRV (70)	**0**	**nd**	**4**	**0**	**0**	**0**	**0**	**0**	**0**
VMIGsPKKV (76)	**0**	**nd**	**0**	**0**	**0**	**0**	**0**	**1**	**0**
IMDRtPEKL (14)	**2**	**nd**	**6**	**0**	**0**	**0**	**2**	**1**	**1**
RVAsPTSGV (57)	**0**	**nd**	**3**	**0**	**0**	**0**	**0**	**0**	**2**
RLAsYLDRV (34)	**0**	**nd**	**nd**	**1**	**0**	**0**	**0**	**0**	**0**
yLQSRYYRA (77)	**1**	**nd**	**nd**	**0**	**0**	**0**	**0**	**2**	**0**
PmVTLsLNL (415)	**0**	**nd**	**nd**	**0**	**0**	**1**	**0**	**2**	**1**
KAFsPVRSV (16)	**0**	**nd**	**nd**	**0**	**0**	**0**	**0**	**0**	**0**
FLDtPIAKV (9)	**0**	**nd**	**nd**	**0**	**1**	**0**	**2**	**0**	**0**

KIAsEIAQL (17)	1	nd	nd	1	1	0	0	0	0
RLSsPLHFV (43)	0	nd	nd	0	0	0	0	0	0
RTFsPTYGL (54)	1	nd	nd	0	0	0	3	0	0
SImsPEIQL (58)	0	nd	nd	0	0	0	2	0	0
ALDsGASLLHL (2)	2	nd	nd	0	0	0	2	0	0
AVVsPPALHNA (6)	2	nd	nd	0	0	0	2	0	0
KLFPDtPLAL (21)	0	nd	nd	nd	nd	nd	0	0	0
RLFsKELRC (39)	0	nd	nd	nd	nd	nd	0	0	0
RQAsIELPSMAV (46)	1	nd	nd	nd	nd	nd	0	0	0
RQDsTPGKVFL (48)	2	nd	nd	nd	nd	nd	1	0	0
RQLsSGVSEI (51)	2	nd	nd	nd	nd	nd	0	0	0

Phospho-Ser, phospho-Thr, and phosphor-Tyr residues are indicated by s, t, and y, respectively. Oxidized methionine residues are depicted by "m".

nd: not determined; 0: <0.25% reactive $CD8^{+}$ T cells; 1: 0.25-2.5% reactive $CD8^{+}$ T cells; 2: 2.5-5.0% reactive $CD8^{+}$ T cells; 3: 5-7.5% reactive $CD8^{+}$ T cells; 4: 7.5-10% reactive $CD8^{+}$ T cells; 5: 10-20% reactive $CD8^{+}$ T cells; 6: >20% reactive $CD8^{+}$ T cells.

Table 24

Summary of ppCTL Responses from pP-specifically Expanded Cultures from "Healthy" Livers, Cirrhotic Livers, and HCCs

Sequence	Deceased Donor Liver				End-stage Liver Cirrhosis				HCC				
									Tumor		Distal		
	Do 2	Do 3	Do 4	Do 6	IHL 11	IHL 12	IHL 13	IHL 15	HCC 1	HCC 9	HCC 1	HCC 4	HCC 9	HCC 12
NLVPMVATV (SEQ ID NO: 451)	3	0	2	0	0	0	0	0	0	0	1	0	0	0
GLCTLVAML (SEQ ID NO: 501)	3	0	3	0	0	0	0	0	0	0	0	0	0	0
RTHsLLLLL (SEQ ID NO: 428)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SMTRsPPRV (SEQ ID NO: 70)	1	0	0	0	0	0	0	6	0	0	0	0	0	0
VMIGsPKKV (SEQ ID NO: 76)	2	0	0	0	0	0	0	0	0	0	0	0	0	0
IMDRtPEKL (SEQ ID NO: 14)	2	0	1	0	0	0	0	0	0	0	0	0	0	0

RVAsPTSGV (SEQ ID NO: 57)	0	0	2	1	0	0	0	0	0	0	0	0
RLAsYLDRV (SEQ ID NO: 34)	0	0	0	0	0	0	0	0	0	0	0	0
yLQSRYYRA (SEQ ID NO: 77)	2	0	0	0	0	0	0	0	1	0	0	0
PmVTLsLNL (SEQ ID NO: 415)	2	0	2	1	0	0	0	0	0	0	0	0
KAFsPVRSV (SEQ ID NO: 16)	1	0	0	0	0	0	0	0	0	0	0	0
FLDtPIAKV (SEQ ID NO: 9)	0	0	0	0	0	0	0	0	0	0	0	0
KIAsEIAQL (SEQ ID NO: 17)	0	0	1	1	0	0	0	0	0	0	0	0
RLSsPLHFV (SEQ ID NO: 43)	0	0	0	0	0	0	0	0	0	0	0	0
RTFsPTYGL (SEQ ID NO: 54)	0	0	0	0	0	0	0	1	0	0	0	0
SImsPEIQL (SEQ ID NO: 58)	0	0	0	0	0	0	0	0	0	0	0	0

ALDsGASLLHL (SEQ ID NO: 2)	0	0	0	0	0	0	3	0	0	0	0	0	0
AVVsPPALHNA (SEQ ID NO: 6)	0	0	0	0	0	0	0	0	1	0	0	0	0
KLFPDtPLAL (SEQ ID NO: 21)	0	0	0	0	nd	0	nd	0	nd	0	0	nd	nd
RLFsKELRC (SEQ ID NO: 39)	1	0	0	0	nd	0	nd	0	nd	0	0	nd	nd
RQAsIELPSMAV (SEQ ID NO: 46)	0	0	0	1	nd	0	nd	0	nd	0	0	nd	nd
RQDsTPGKVFL (SEQ ID NO: 48)	1	0	0	0	nd	0	nd	0	nd	0	0	nd	nd
RQLsSGVSEI (SEQ ID NO: 51)	0	0	0	0	nd	0	nd	0	nd	0	0	nd	nd

Phospho-Ser, phospho-Thr, and phosphor-Tyr residues are indicated by s, t, and y, respectively. Oxidized methionine residues are depicted by "m".

nd: not determined; 0: <0.25% reactive $CD8^{-+}$ T cells; 1: 0.25-2.5% reactive $CD8^{-+}$ T cells; 2: 2.5-5.0% reactive $CD8^{-+}$ T cells; 3: 5-7.5% reactive $CD8^{-+}$ T cells; 4: 7.5-10% reactive $CD8^{-+}$ T cells; 5: 10-20% reactive $CD8^{-+}$ T cells; 6: >20% reactive $CD8^{-+}$ T cells.

Table 25

Functional Annotation Clustering of MHC-I-pP to Biological Processes

(GO Term BP) using DAVID v6.7

Cluster rank	GO Term BP cluster	Included GO Terms	Proteins in cluster	Enrichment Score
1	Phosphorylation	GO:0006468; GO:0016310; GO:0006793; GO:0006796	22	3.10
2	Negative regulation of transcription	GO:0051253; GO:0010605; GO:0006357; GO:0045892; GO:0051172; GO:0010629; GO:0045934; GO:0016481; GO:0009890; GO:0051252; GO:0010558; GO:0045449; GO:0031327; GO:0006350; GO:0000122; GO:0006355.	45	2.57
3	Cell cycle	GO:0007049; GO:0022403; GO:0000279; GO:0022402; GO:0000278; GO:0051301; GO:0000280; GO:0007067; GO:0000087; GO:0048285.	22	2.48
4	Apoptosis / Cell Death	GO:0008219; GO:0016265; GO:0006915; GO:0012501 GO:0042981; GO:0043067; GO:0010941	20	2,46
5	Transcription RNA Pol II	GO:0006366; GO:0006351; GO:0032774	9	1.82

6	Regulation of metabolic processes	GO:0032268; GO:0031399; GO:0044093; GO:0001932; GO:0046328; GO:0080135; GO:0045859; GO:0070302; GO:0042325; GO:0043506; GO:0043549; GO:0019220; GO:0051174; GO:0051338; GO:0043408; GO:0043085; GO:0010627; GO:0045860; GO:0033674; GO:0032147; GO:0051347; GO:0043405	17	1.79
7	Apoptosis / Cell Death	GO:0042981; GO:0043067; GO:0010941; GO:0043065; GO:0043068; GO:0010942; GO:0006917; GO:0012502; GO:0008624	19	1.635
8	Apoptosis	GO:0042981; GO:0043067; GO:0010941; GO:0043066; GO:0043069; GO:0060548; GO:0006916	19	1.54
9	Cell cycle / mitosis	GO:0000278; GO:0051329; GO:0051325; GO:0000079	12	1.54
10	Metabolic processes / biosythesis	GO:0009891; GO:0031328; GO:0010557; GO:0010604; GO:0045893; GO:0051254; GO:0045941; GO:0051173; GO:0010628; GO:0045935; GO:0045944	18	1.51
11	Cytoskeleton organization	GO:0007010; GO:0030036; GO:0030029	14	1.47
12	DNA repair	GO:0006974; GO:0033554; GO:0006281; GO:0006259; GO:0006260	15	1.45
13	Myeloid cell differentiation	GO:0045639; GO:0002763; GO:0045637; GO:0045597; GO:0002761; GO:0051094	7	1.42

14	Response to hormone stimulus (e.g. insulin)	GO:0032870; GO:0010033; GO:0032869; GO:0009725; GO:0009719; GO:0032868; GO:0007169; GO:0043434	9	1.40
15	Cell cycle / mitosis	GO:0045859; GO:0043549; GO:0051338; GO:0000280; GO:0007067; GO:0000087; GO:0048285; GO:0045786; GO:0006469; GO:0033673; GO:0051348; GO:0044092; GO:0043086; GO:0010498; GO:0043161	11	1.32
16	DNA binding	GO:0043388; GO:0051101; GO:0051099; GO:0051098; GO:0051090	5	1.04
17	RNA catabolic process	GO:0000956; GO:0006402; GO:0006401	3	1.00
18	RNA splicing / processing	GO:0016071; GO:0006397; GO:0006396; GO:0008380	10	0.98
19	Protein modification	GO:0031399; GO:0031401; GO:0032270; GO:0051247	11	0.89
20	Protein complex assembly / biogenesis	GO:0070271; GO:0006461; GO:0043933; GO:0065003	12	0.74
21	Chromatin organization	GO:0006325; GO:0051276; GO:0016568	9	0.56
22	Membrane organization	GO:0016044; GO:0016192; GO:0010324; GO:0006897	9	0.56
23	Cell motility / migration	GO:0006928; GO:0048870; GO:0051674; GO:0016477	9	0.54

Table 26

Biocarta and KEGG Signaling Pathway Mapping of Identified MHC-I-pP Revealed that MHC-I-pP were Significantly Enriched in the MAPK Signaling Pathway

Signaling pathway	Proteins	Database	*p* value	Significance for cancer
MAPKinase	DAXX, HSPB1, JUN, MAP3K11, MAP3K3, RPS6KA1, RAF1	Biocarta	0.012	MAPK pathways are evolutionarily conserved kinases that link extracellular signals to the machinery that controls fundamental cellular processes such as growth, proliferation, differentiation, migration and apoptosis.
		KEGG	0.1	
		BBID	0.074	
Neurotrophin	RAPGEF1, IRS2, JUN, MAP3K3, RPS6KA1, RAF1	KEGG	0.017	Neurotrophins play a major role in neuron survival, proliferation, differentiation and apoptosis. They function by interacting with tyrosine kinase receptors which activate MAPK, PI3K and PLC-γ pathways.

Table 27

Exemplary Phosphopeptides Found on >1 Sample or Cancer**

Gene (UniProt Acc. No.)	Phosphopeptide	HLA-motif%	Found on	Other cancers	Function and significance for cancer
SRSF7 (Q16629)	SPRRsRSISL (SEQ ID NO: 284)	B*07	LL4857T	CRC, Mel, BCa, HaemCa	Required for pre-mRNA splicing.
SRSF8 (Q9BRL6)	SMTRsPPRV (SEQ ID NO: 70)	A*02	HepG2	CRC, OEAC, Mel, OvCa	Involved in pre-mRNA alternative splicing.
LATS1 (O95835)	KRYsGNmEY (SEQ ID NO: 242)	C*07	LL3907T LL370T LL981T LL4857T	CRC, OEAC, Mel	Negative regulator of YAP1 in the Hippo signalling pathway. Acts as a tumor suppressor in controlling mitotic progression. Involved in the control of p53 expression.
LATS2 (Q9NRM7)	RRDsLQKPGL (SEQ ID NO: 248)	B*27	LL981T	CRC	Same as for LATS1.

BRD4 (O60885)	REAPsPLmI (SEQ ID NO: 352)	B*49	LL3907T	HaemCa	Recognizes and binds acetylated histones and plays a key role in transmission of epigenetic memory across cell divisions and transcription regulation. Also acts as a regulator of p53/TP53-mediated transcription.
	AVVsPPALHNA (SEQ ID NO: 6)	A*02	LL981 HepG2	OvCa, HaemCa	
SIPA1L1 (O43166)	REYGsTSSI (SEQ ID NO: 364)	B*49	LL3907T	-	Promotes reorganization of the actin cytoskeleton.
	RRPsYTLGM (SEQ ID NO: 273)	C*07	LL370T	Mel	
SIPA1L3 (O60292)	REVsPAPAV (SEQ ID NO: 363)	B*49	LL3907T	-	?
HIPK3 (Q9H422)	yLQSRYYRA (SEQ ID NO: 77)	A*02	LL3907T LL4857T LL981T	OvCa	Kinase involved in transcription regulation, apoptosis and steroidogenic gene expression. Phosphorylates JUN and RUNX2.
HIPK1 (Q86Z02)	YPLsPTKISQY (SEQ ID NO: 296)	B*35	HepG2	OEAC	Serine/threonine-protein kinase involved in transcription regulation and TNF-mediated cellular apoptosis. Activates nuclear MAP3K5-JNK. May be involved in anti-oxidative stress responses. Involved in the regulation of eye embryogenesis. Promotes angiogenesis. May be involved in malignant squamous cell tumor formation.

EPN1 (Q9Y613)	TRKtPESFL (SEQ ID NO: 381)	C*06	LL981T	CRC, OEAC	Modifies membrane curvature and facilitates the formation of clathrin-coated invaginations. Regulates receptor-mediated endocytosis
	RPIsPRIGAL (SEQ ID NO: 141)	B*07	LL4857T	CRC, OEAC, BCa, HaemCa	
NCOR1 (O75376)	SARRRtPVSY (SEQ ID NO: 529)	C*02	LL981T	-	Mediates transcriptional repression by certain nuclear receptors. Part of a complex which promotes histone deacetylation and the formation of repressive chromatin structures which may impede the access of basal transcription factors
	RRPsLLSEF (SEQ ID NO: 271)	B*27	LL981N +T	CRC, OEAC	
NCOR2 (Q9Y618)	ITQGtPLKY (SEQ ID NO: 289)	A*01	LL981T	CRC, OEAC, Mel, HaemCa	Transcriptional corepressor. Involved in the regulation of the germinal centre B-cells proliferation and survival.
MAP3K3 (Q99759)	GRsSPPPGY (SEQ ID NO: 232)	B*27	LL981T	-	Component of a MAP kinase signal transduction cascade. Mediates activation of the NF-kappa-B, AP1 and DDIT3 transcriptional regulators.
MAP3K11 (Q16584)	TPRsPPLGLI (SEQ ID NO: 217)	B*07	LL4857T	Mel, BCa, HaemCa, Mel, CRC	Activates the JUN N-terminal pathway. Plays a role in mitogen-stimulated activation of BRAF. Influences microtubule organization during the cell cycle.

GAREM (Q9H706)	LLDPSRSYsY (SEQ ID NO: 290)	A*01	LL981T	Mel	Acts as an adapter protein that plays a role in intracellular signalling cascades triggered either EGFR and/or cytoplasmic protein tyrosine kinases. Promotes activation of the MAPK/ERK signalling pathway. Plays a role in the regulation of cell proliferation.
	RSYsYPRQK (SEQ ID NO: 326)	A*03	LL4857T	Mel	
ZFP36L1 (Q07352)	GLLDsPTSI (SEQ ID NO: 13)	A*02	LL370T	OvCa	Probable regulatory protein involved in regulating the response to growth factors.
ZFP36L2 (P47974)	RRHsASNLHAL (SEQ ID NO: 375)	C*06	L981T	-	mRNA-binding protein that plays a key role in self-renewal of erythroid cells in response to glucocorticoids.
PRKAR1A (P10644)	RPRAAtVV (SEQ ID NO: 153)	B*07	LL4857T	CRC	Regulatory subunit of the cAMP-dependent protein kinases involved in cAMP signaling in cells. Membrane association by binding to anchoring proteins, including the MAP2 kinase.
PRKAR2A (P13861)	SRFNRRVsV (SEQ ID NO: 285)	C*06	LL981T	-	
ANAPC1 (Q9H1A4)	VLLsPVPEL (SEQ ID NO: 74)	A*02	HepG2	Mel, HaeCa,	Component of the anaphase promoting complex/cyclosome (APC/C), a cell cycle-regulated E3 ubiquitin ligase that controls progression through mitosis and the G1 phase of the cell cycle.
ANAPC7 (Q9UJX3)	KRYsRALYL (SEQ ID NO: 243)	C*07	LL370T	OvCa, CRC, OEAC, Mel	

MEF2D (Q14814)	RLLsPQQPAL (SEQ ID NO: 122)	A*02	LL370T	BCa	Transcriptional activator mediates cellular functions in muscle development, but also in neuronal differentiation and survival. Plays diverse roles in the control of cell growth, survival and apoptosis via p38 MAPK signalling.
	RPAsAGAmL (SEQ ID NO: 125)	B*07	LL4857T	CRC, HaemCa, Mel	
AKAP13 (Q12802)	RRSFsLE (SEQ ID NO: 275)	?	LL370T	-	Anchors cAMP-dependent protein kinase (PKA). Augments gene activation by the estrogen receptor and activates estrogen receptor beta by a p38 MAPK-dependent pathway.
	RPRsAVLL (SEQ ID NO: 169)	B*07	LL4857N+T	CRC, BCa	
MICALL2 (Q8IY33)	KLPsPAPARK (SEQ ID NO: 308)	A*03	LL4857N+T	CRC	Regulates cell adhesion molecules transport to the plasma membrane and actin cytoskeleton reorganization. Most probably involved in the processes of epithelial cell differentiation, cell spreading and neurite outgrowth.
	RPAsPGPSL (SEQ ID NO: 128)	B*07	LL4857T	BCa	
MICAL3 (Q7RTP6)	RPDsPTRPTL (SEQ ID NO: 133)	B*07	LL4857T	BCa	Acts by modifying actin subunits, leading to promote actin filament severing and prevent repolymerization.
BCAR3 (O75815)	RPDVAKRLsL (SEQ ID NO: 135)	B*07	LL4857T	CRC	May act as an adapter protein and couples activated growth factor receptors to a signalling pathway that regulates the proliferation in breast cancer cells. When overexpressed, it confers anti-estrogen resistance in breast cancer cell lines. May also be regulated by cellular adhesion to extracellular matrix proteins.
	IMDRfPEKL (SEQ ID NO: 14)	A*02	LL4857T	Mel, Haem Ca, OvCa	

TP53BP1 (Q12888)	SIDsPQKL (SEQ ID NO: 388)	C*05	LL4857T	Mel, CRC	Plays a key role in the response to DNA damage. May have a role in checkpoint signaling during mitosis. Enhances TP53-mediated transcriptional activation.
	RSDsYVEL (SEQ ID NO: 385)	C*05	LL4857T	CRC	

** Many of these MHC-I-pP are in a central position of essential cancer-associated signaling pathways.

% Predicted.

Table 28

Phosphopeptides Identified in HCC and Other Cancer Types Sorted by HLA Type

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
286	AEQGsPRVSY	A*0101						
287	GsPHYFSPFRPY	A*0101						
288	ISSsMHSLY	A*0101		✓				
289	ITQGtPLKY	A*0101		✓	✓			✓
290	LLDPSRSYsY	A*0101			✓			
291	SLDsPSYVLY	A*0101			✓			
292	SLYDRPAsY	A*0101						
293	SYPsPVATSY	A*0101						
294	TMAsPGKDNY	A*0101		✓	✓			✓
295	YFsPFRPY	A*0101						
296	YPLsPTKISQY	A*0101						✓
297	YQRPFsPSAY	A*0101						
1	AIMRsPQMV	A*0201				✓		
2	ALDsGASLLHL	A*0201		✓		✓		
3	ALGNtPPFL	A*0201				✓		
4	ALMGsPQLV	A*0201				✓		
5	ALMGsPQLVAA	A*0201				✓		
6	AVVsPPALHNA	A*0201			✓	✓		
7	DLKRRsmSI	A*0201		✓				
8	ELFSsPPAV	A*0201				✓		
9	FLDtPIAKV	A*0201		✓				
10	GIDsPSSSV	A*0201						
11	GLDsGFHSV	A*0201						
12	GLIsPVWGA	A*0201				✓		
13	GLLDsPTSI	A*0201				✓		
14	IMDRtPEKL	A*0201			✓	✓		
404	IQFsPPFPGA	A*0201						
15	KAFsPVR	A*0201		✓		✓		
16	KAFsPVRSV	A*0201		✓		✓		✓
17	KIAsEIAQL	A*0201				✓		

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
18	KIGsIIFQV	A*0201				✓		
19	KLAsPELERL	A*0201		✓	✓	✓		
20	KLDsPRVTV	A*0201				✓		
21	KLFPDtPLAL	A*0201		✓	✓	✓		
22	KLIDIVsSQKV	A*0201		✓	✓	✓		
23	KLIDRTEsL	A*0201		✓		✓		
409	KLKDRLPsI	A*0201						
24	KLMsDVEDV	A*0201				✓		
25	KLMsPKADVKL	A*0201			✓	✓		
410	KLsGDQPAAR	A*0201						
26	KQDsLVINL	A*0201				✓		
27	KTMsGTFLL	A*0201		✓		✓		
28	KVAsLLHQV	A*0201				✓		
29	LMFsPVTSL	A*0201			✓	✓		
415	PmVTLsLNL	A*0201						
30	RASsLSITV	A*0201				✓		
31	RLAsASRAL	A*0201				✓		
32	RLAsLQSEV	A*0201				✓		
33	RLAsYLDKV	A*0201				✓		
34	RLAsYLDRV	A*0201		✓				
35	RLDsYVR	A*0201			✓	✓		
36	RLDsYVRSL	A*0201			✓	✓		✓
37	RLFsKEL	A*0201						
38	RLFsKELR	A*0201			✓	✓		
39	RLFsKELRC	A*0201			✓	✓		
40	RLLsDLEEL	A*0201				✓		
41	RLLsTDAEAV	A*0201				✓		
42	RLSDtPPLL	A*0201				✓		
43	RLSsPLHFV	A*0201		✓	✓	✓		
44	RMYsFDDVL	A*0201				✓		
45	RQAsIELPSM	A*0201	✓	✓				
46	RQAsIELPSMAV	A*0201		✓				

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
47	RQAsLSISV	A*0201				✓		
48	RQDsTPGKVFL	A*0201		✓	✓	✓		
49	RQIsQDVKL	A*0201		✓	✓	✓		✓
50	RQLsALHRA	A*0201				✓		
51	RQLsSGVSEI	A*0201		✓	✓	✓		
52	RSLsESYEL	A*0201				✓		
53	RSLsQELVGV	A*0201				✓		
54	RTFsPTYGL	A*0201			✓	✓		✓
55	RTLsHISEA	A*0201			✓	✓		
56	RTYsGPMNKV	A*0201				✓		
57	RVAsPTSGV	A*0201		✓	✓	✓		
58	SImsPEIQL	A*0201				✓		
59	SISsMEVNV	A*0201		✓				
60	SISStPPAV	A*0201				✓		
61	SLFGGsVKL	A*0201				✓		
62	SLFsGDEENA	A*0201				✓		
63	SLFsPQNTL	A*0201				✓		
64	SLFsSEESNL	A*0201				✓		
65	SLFsSEESNLGA	A*0201			✓	✓		
66	SLHDIQLsL	A*0201				✓		
67	SLQPRSHsV	A*0201				✓		
68	SLQsLETSV	A*0201				✓		
69	SMSsLSREV	A*0201				✓		
70	SMTRsPPRV	A*0201			✓	✓		
71	SVKPRRTsL	A*0201		✓				
72	TVFsPTLPAA	A*0201				✓		
443	VLFPEsPARA	A*0201						
73	VLFSsPPQM	A*0201				✓		
444	VLIENVAsL	A*0201						
74	VLLsPVPEL	A*0201			✓	✓		
445	VLSDVIPsI	A*0201						
446	VLVVDTPsI	A*0201						

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
75	VLYsPQMAL	A*0201				✓		
76	VMIGsPKKV	A*0201		✓	✓	✓		
77	yLQSRYYRA	A*0201				✓		
298	ATYtPQAPK	A*0301			✓			
299	FLIIRtVLQL	A*0301						
300	FRYsGKTEY	A*0301			✓			
301	GIMsPLAKK	A*0301	✓	✓				
402	HTAsPTGMMK	A*0301						
403	HVYtPSTTK	A*0301						
302	IISsPLTGK	A*0301			✓			
303	ILKPRRsL	A*0301						
304	IYQyIQSRF	A*0301	✓	✓	✓			
305	KLPDsPALA	A*0301						
306	KLPDsPALAK	A*0301			✓			
307	KLPDsPALAKK	A*0301			✓			
308	KLPsPAPARK	A*0301		✓				
309	KLRsPFLQK	A*0301	✓		✓			
310	KMPTtPVKAK	A*0301		✓	✓			
311	KRAsVFVKL	A*0301			✓			
312	KTPTsPLKMK	A*0301	✓		✓			
313	KVQsLRRAL	A*0301		✓	✓			
314	MTRsPPRVSK	A*0301			✓			
315	RAKsPISLK	A*0301	✓	✓	✓			
419	RIGsPLSPK	A*0301						
316	RILsGVVTK	A*0301	✓	✓	✓			
317	RIYQyIQ	A*0301		✓	✓			
318	RIYQyIQSR	A*0301		✓	✓			
319	RIYQyIQSRF	A*0301		✓	✓			
320	RLFVGsIPK	A*0301						
321	RLLDRSPsRSAK	A*0301			✓			
322	RLSsPISKR	A*0301	✓	✓	✓			
323	RLSsPVLHR	A*0301			✓			

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
424	RMFsPMEEK	A*0301						
324	RSLsVEIVY	A*0301			✓			
325	RSYsRSFSR	A*0301			✓			
326	RSYsYPRQK	A*0301			✓			
327	RTAsFAVRK	A*0301			✓			
328	RTAsPPPPPK	A*0301	✓		✓			
429	RTNsPGFQK	A*0301						
329	RTRsLSSLREK	A*0301		✓				
432	RTSsPLFNK	A*0301						
433	RTYsHGTYR	A*0301						
330	RVAsPTSGVK	A*0301		✓	✓			
331	RVKtPTSQSYR	A*0301			✓			
332	RVLsPLIIK	A*0301		✓	✓			
333	RVRQsPLATR	A*0301		✓	✓			
334	RVYsPYNHR	A*0301	✓	✓	✓			
335	SVKsPVTVK	A*0301		✓	✓			
336	SVRRsVLMK	A*0301		✓	✓			
441	TLLAsPMLK	A*0301						
337	yIQSRF	A*0301			✓			
416	PYDPALGsPSR	A*24						
389	RYQtQPVTL	A*24		✓				
390	VYTyIQSRF	A*24						
399	FTKsPYQEF	A*26						
391	RTSsFTFQN	A*31		✓				
78	APDsPRAFL	B*0702		✓				
79	APRKGsFSAL	B*0702		✓	✓		✓	
80	APRNGsGVAL	B*0702						
81	APRRYsSSL	B*0702	✓	✓	✓			✓
82	APRsPPPSRP	B*0702			✓			
83	APSLFHLNtL	B*0702						
84	APSSARAsPLL	B*0702						
85	FPLDsPKTLVL	B*0702						

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
86	FPRRHsVTL	B*0702	✓	✓	✓			
87	FRGRYRsPY	B*0702		✓				
88	FRKsMVEHY	B*0702			✓			
89	GPPYQRRGsL	B*0702						
90	GPRPGsPSAL	B*0702			✓		✓	
91	GPRSAsLL	B*0702	✓	✓	✓			
92	GPRSAsLLSL	B*0702	✓	✓	✓			
93	GPRSAsLLsL	B*0702	✓					
94	GPRsPKAPP	B*0702	✓	✓	✓			
95	HPKRSVsL	B*0702			✓			
96	HRYsTPHAF	B*0702		✓				
405	KASPKRLsL	B*0702						
411	KLSGLsF	B*0702	✓					
97	KPAsPKFIVTL	B*0702	✓	✓	✓			
98	KPPYRSHsL	B*0702	✓					
99	KPRPLsMDL	B*0702		✓				
100	KPRPPPLsP	B*0702		✓	✓			
101	KPRRFsRsL	B*0702	✓	✓	✓			
101	KPRRFsRSL	B*0702	✓	✓	✓			
102	KPRsPFSKI	B*0702		✓				
103	KPRsPPRAL	B*0702	✓	✓	✓		✓	
104	KPRsPPRALVL	B*0702			✓		✓	
105	KPRsPVVEL	B*0702	✓	✓	✓		✓	✓
106	KPSsPRGSL	B*0702						
107	KPSsPRGSLL	B*0702						
108	KPVsPKSGTL	B*0702	✓		✓			
109	KPYsPLASL	B*0702	✓	✓	✓			
110	KRAsGQAFEL	B*0702			✓			
111	LPAsPRARL	B*0702	✓	✓	✓		✓	✓
112	LPIFSRLsI	B*0702		✓	✓		✓	
113	LPKGLSAsL	B*0702						
113	LPKGLsASL	B*0702						

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
114	LPRGsSPSVL	B*0702	✓	✓	✓		✓	
115	LPRPAsPAL	B*0702						
116	LPRSSsMAA	B*0702						
117	LPRSSsMAAGL	B*0702		✓				
118	MPRQPsATRL	B*0702	✓	✓	✓			
119	QPRtPSPLVL	B*0702	✓	✓	✓			
120	RARGIsPIVF	B*0702	✓	✓	✓		✓	
121	RKLsVILIL	B*0702		✓	✓			
122	RLLsPQQPAL	B*0702					✓	
123	RPAFFsPSL	B*0702	✓		✓			
124	RPAKsMDSL	B*0702	✓	✓	✓			
125	RPAsAGAmL	B*0702	✓	✓	✓			
126	RPAsPAAKL	B*0702	✓	✓	✓		✓	
127	RPAsPEPEL	B*0702						
128	RPAsPGPSL	B*0702						
129	RPAsPQRAQL	B*0702	✓	✓	✓		✓	
130	RPAsPSLQL	B*0702		✓				
131	RPAsPSLQLL	B*0702		✓				
132	RPAsYKKKSML	B*0702						
133	RPDsPTRPTL	B*0702		✓				
134	RPDsRLGKTEL	B*0702	✓	✓	✓		✓	
135	RPDVAKRLsL	B*0702		✓				
136	RPFHGISTVsL	B*0702		✓				
137	RPFsPREAL	B*0702	✓	✓	✓		✓	✓
138	RPGsRQAGL	B*0702		✓				
139	RPIsPGLSY	B*0702		✓	✓		✓	✓
140	RPIsPPHTY	B*0702						✓
141	RPIsPRIGAL	B*0702		✓				
142	RPKLSsPAL	B*0702	✓	✓	✓			
143	RPKsNIVLL	B*0702						
144	RPKsPLSKM	B*0702		✓				
145	RPKsVDFDSL	B*0702		✓	✓			

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
146	RPKtPPVVI	B*0702	✓	✓	✓			
147	RPLsLLLAL	B*0702						
148	RPLsVVYVL	B*0702						
149	RPMsESPHM	B*0702						✓
150	RPNsPSPTAL	B*0702	✓		✓		✓	
151	RPPsPGPVL	B*0702	✓		✓			
152	RPQRAtSNVF	B*0702	✓	✓	✓		✓	
153	RPRAAtVV	B*0702		✓				
154	RPRAAtVVA	B*0702	✓	✓				
155	RPRANsGGVDL	B*0702	✓	✓	✓			
156	RPRARsVDAL	B*0702		✓	✓		✓	✓
157	RPRDtRRISL	B*0702	✓					
158	RPRGsESLL	B*0702						
159	RPRGsQSLL	B*0702		✓				✓
160	RPRIPsPIGF	B*0702	✓		✓			
161	RPRPAsSPAL	B*0702						
162	RPRPHsAPSL	B*0702	✓	✓	✓			
163	RPRPSsAHVGL	B*0702		✓				
164	RPRPsSVL	B*0702						
165	RPRPsSVLRTL	B*0702						
166	RPRPVsPSSL	B*0702	✓	✓	✓		✓	✓
167	RPRPVsPSSLL	B*0702			✓			
168	RPRsAVEQL	B*0702		✓				
169	RPRsAVLL	B*0702	✓	✓	✓			
170	RPRsISVEEF	B*0702		✓	✓			
171	RPRsLEVTI	B*0702	✓					
172	RPRSLsSPTVTL	B*0702			✓		✓	
173	RPRsMTVSA	B*0702	✓	✓				
174	RPRsMVRSF	B*0702						
175	RPRsPAARL	B*0702	✓					
176	RPRsPNMQDL	B*0702		✓				
177	RPRsPPGGP	B*0702		✓				

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
178	RPRsPPPRAP	B*0702		✓	✓			
179	RPRsPPSSP	B*0702	✓		✓			
180	RPRsPRENSI	B*0702	✓	✓	✓		✓	
181	RPRsPRPPP	B*0702			✓			
182	RPRsPRQNSI	B*0702	✓	✓	✓		✓	✓
183	RPRSPsPIS	B*0702						
184	RPRsPTGPSNSF	B*0702		✓	✓			
185	RPRsPTGPSNSFL	B*0702			✓			
186	RPRsPWGKL	B*0702						
187	RPRsQYNTKL	B*0702						
188	RPRtPLRSL	B*0702			✓			
189	RPSsLPDL	B*0702	✓		✓		✓	
190	RPSsPALYF	B*0702	✓					
191	RPTsFADEL	B*0702						✓
192	RPTsRLNRL	B*0702	✓	✓	✓		✓	
193	RPVsPFQEL	B*0702	✓	✓	✓		✓	✓
194	RPVsPGKDI	B*0702	✓		✓		✓	
195	RPVSPsSLL	B*0702			✓			
196	RPVsTDFAQY	B*0702						✓
197	RPVtPVSDL	B*0702	✓	✓	✓			
198	RPWsNSRGL	B*0702						
199	RPWsPAVSA	B*0702	✓	✓			✓	
200	RPYsPPFFSL	B*0702	✓		✓			
201	RPYsQVNVL	B*0702						
202	RSRsPRPAL	B*0702			✓	✓		
203	RTRsPSPTL	B*0702			✓			
431	RVRKLPsTTL	B*0702	✓		✓			
204	SPAsPKISL	B*0702	✓		✓		✓	
205	SPFKRQLsL	B*0702		✓	✓		✓	✓
206	SPFLsKRSL	B*0702						
207	SPGLARKRsL	B*0702	✓					
208	SPKsPGLKA	B*0702	✓	✓	✓			

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
209	SPRERsPAL	B*0702		✓	✓		✓	
210	SPRGEASsL	B*0702						
210	SPRGEAsSL	B*0702						
211	SPRsPGRSL	B*0702	✓		✓		✓	
212	SPRsPSGLR	B*0702						
213	SPRSPsTTYL	B*0702	✓	✓	✓			
214	SPSsPSVRRQL	B*0702	✓		✓			
215	TPMKKHLsL	B*0702						
216	TPRsPPLGL	B*0702	✓	✓	✓		✓	✓
217	TPRsPPLGLI	B*0702	✓	✓	✓		✓	
218	VAKRLsL	B*0702						
219	VPRPERRsSL	B*0702		✓				
220	VPRsPKHAHSSSL	B*0702	✓	✓				
221	VPTsPKSSL	B*0702					✓	
222	YPDPHsPFAV	B*0702						
223	YPGGRRsSL	B*0702		✓				
224	YPYEFsPVKM	B*0702						
398	DLKSSKAsL	B*08						
438	SsPIMRKKVSL	B*08						
400	GQLsPGVQF	B*1508						
108	KIKsFEVVF	B*1508						
392	RAHsEPLAL	B*1508	✓					
225	FRRsPTKSSL	B*2705	✓	✓				
226	FRRsPTKSSLD	B*2705	✓	✓				
227	FRRsPTKSSLDY	B*2705	✓	✓				
228	GRKsPPPSF	B*2705		✓				
229	GRLsPAYSL	B*2705		✓				
230	GRLsPVPVPR	B*2705		✓				
231	GRQsPSFKL	B*2705						
232	GRsSPPPGY	B*2705						
233	KRAsYILRL	B*2705						
234	KRFsFKKSF	B*2705		✓				

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
235	KRFsFKKsF	B*2705		✓	✓			
236	KRFsGTVRL	B*2705		✓	✓			
237	KRKsFTSLY	B*2705						
238	KRLEKsPSF	B*2705						
239	KRLsPAPQL	B*2705	✓	✓	✓			
240	KRmsPKPEL	B*2705		✓	✓			
241	KRWQsPVTK	B*2705						
242	KRYsGNmEY	B*2705		✓	✓			✓
243	KRYsRALYL	B*2705						
244	QRLsPLSAAY	B*2705						
420	RKLRsLEQL	B*2705						
245	RRAsIITKY	B*2705						
246	RRAsLSEIGF	B*2705		✓				
247	RRDsIVAEL	B*2705		✓				
248	RRDsLQKPGL	B*2705		✓				
249	RRFsGTAVY	B*2705	✓	✓				✓
250	RRFsIATLR	B*2705		✓				
251	RRFsLTTLR	B*2705		✓				
252	RRFsPPRRm	B*2705	✓	✓				✓
253	RRFsRSDEL	B*2705						
254	RRFsRsPIR	B*2705						
255	RRFSRsPIR	B*2705			✓			
256	RRFsRsPIRR	B*2705			✓			
257	RRGsFEVTL	B*2705		✓				✓
258	RRIDIsPSTF	B*2705		✓				
259	RRIsDPEVF	B*2705	✓	✓				✓
260	RRIsDPQVF	B*2705		✓				
261	RRIsQIQQL	B*2705						
262	RRKsQVAEL	B*2705	✓	✓	✓			✓
263	RRLsADIRL	B*2705		✓	✓			
264	RRLsELLRY	B*2705		✓				
265	RRLsGGSHSY	B*2705			✓			

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
266	RRLsRKLSL	B*2705						
267	RRMsFQKP	B*2705		✓				
268	RRmsLLSVV	B*2705		✓	✓			✓
269	RRNsAPVSV	B*2705		✓				✓
270	RRPsIAPVL	B*2705						
271	RRPsLLSEF	B*2705		✓				✓
272	RRPsLVHGY	B*2705			✓			
273	RRPsYTLGM	B*2705			✓			
274	RRRsLERLL	B*2705						
275	RRSFsLE	B*2705						
276	RRSsFLQ	B*2705						
277	RRSsFLQVF	B*2705		✓	✓			
278	RRSsIQSTF	B*2705		✓				
279	RRSsQSWSL	B*2705	✓	✓	✓			
280	RRVVQRSsL	B*2705			✓			
281	RRYsKFFDL	B*2705						
282	RRYsPPIQR	B*2705	✓		✓			
283	RSRsPLEL	B*2705			✓			
284	SPRRsRSISL	B*2705	✓	✓	✓	✓		
285	SRFNRRVsV	B*2705						
397	DAKKsPLAL	B*35						
436	SDmPRAHsF	B*37						
338	AENARSAsF	B*4402						
339	AENsPTRQQF	B*4402	✓	✓	✓			
340	AENsSSREL	B*4402						
341	AtAGPRLGW	B*4402			✓			
342	EELsPTAKF	B*4402	✓	✓				
343	FKtQPVTF	B*4402		✓				
344	GEAsPSHII	B*4402						
345	GEIsPQREV	B*4402						
346	GETsPRTKI	B*4402						
347	HEKKAYsF	B*4402						

SEQ ID NO:	Sequence#	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
348	KEKsPFRET	B*4402						
349	KELARQIsF	B*4402						
350	KEmsPTRQL	B*4402		✓	✓			
351	KESsPLSSRKI	B*4402						
352	REAPsPLmI	B*4402						
352	REAPsPLmI	B*4402						
353	REAsPAPLA	B*4402						
354	REAsPRLRV	B*4402						
355	REAsPSRLSV	B*4402						
356	REIMGtPEYL	B*4402						
357	REKsPGRmL	B*4402						
358	RELARKGsL	B*4402						
359	RELsPLISL	B*4402						
360	REPsPLPEL	B*4402						
361	RERsPSPSF	B*4402						
362	RESsPTRRL	B*4402						
363	REVsPAPAV	B*4402						
364	REYGsTSSI	B*4402						
365	RFKtQPVTF	B*4402		✓				
366	RQKsPLFQF	B*4402			✓			
367	SEFKAMDsI	B*4402						
368	SELsPGRSV	B*4402						
369	TEAsPESML	B*4402						
370	YEGsPIKV	B*4402	✓					
393	ADLsPEREV	B*49						
437	SFDsGSVRL	C*04						
394	AGDsPGSQF	C*0501						
383	KVDsPVIF	C*0501						
413	NMDsPGPML	C*0501						
384	RADsPVHM	C*0501	✓	✓				
422	RLLDPsSPLAL	C*0501						
422	RLLDPSsPLAL	C*0501						

SEQ ID NO:	Sequence[#]	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
385	RSDsYVEL	C*0501	✓	✓				✓
386	RSEsPPAEL	C*0501						
387	RVDsPSHGL	C*0501				✓		
435	sDDEKMPDLE	C*0501						
388	SIDsPQKL	C*0501		✓	✓			
447	VVDsPGQEVL	C*0501						
371	FRFsGRTEY	C*0602						
372	KRAsFAKSV	C*0602	✓		✓			
373	LSSsVIREL	C*0602						
374	RKPsIVTKY	C*0602	✓					✓
375	RRHsASNLHAL	C*0602						
376	RRLsFLVSY	C*0602		✓	✓			✓
377	RRLsYVLFI	C*0602						
378	RRPsYRKIL	C*0602			✓			
379	RSAsFSRKV	C*0602						
380	SRSSSVLsL	C*0602						✓
381	TRKtPESFL	C*0602						✓
382	YRYsPQSFL	C*0602						
426	RNLsSPFIF	C*07						
431	RTSsFALNL	C*07						
442	TLMERTVsL	C*07						
412	KTMsPSQMIM	C*16						
425	RMYsPIPPSL	C*16						
430	RTPsDVKEL	C*16						
448	YARsVHEEF	C*16						
395	AKLsETIS	-----						
396	AsLGFVF	-----						
401	GsPHYFSPF	-----						
406	KAVsLFLcY	-----						
417	RAFsVKFEV	-----						
418	RGDGYGtF	-----						
421	RKSsIIIRM	-----						

SEQ ID NO:	Sequence[#]	HLA-Type	Leukemia	Colorectal	Melanoma	Ovarian	Breast	Esophageal
423	RLSsLRASTSK	-----						
428	RTHsLLLLL	-----	✓	✓		✓		✓
434	RYPsNLQLF	-----						
439	sYIEHIFEI	-----						
440	sYQKVIELF	-----						

[#] Phospho- Ser, -Thr, and -Tyr residues are indicated by "s", "t", and "y", respectively. A lowercase "c" in a peptide sequence indicates that in some embodiments the cysteine is present in a cysteine-cysteine disulfide bond at the surface of a cell and, in some embodiments, is presented to the immune system as such. A lowercase "m" in a peptide sequence indicates that in some embodiments the methionine is oxidized. The presence of phosphopeptides in previously analyzed samples including leukemia, colorectal cancer, melanoma, ovarian cancer, breast cancer, and esophageal cancer is indicated by ✓. White boxes indicate instances in which the phosphopeptide is unique to liver samples.

CLAIMS

What is claimed is:

1. A composition comprising at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more synthetic target peptides, wherein each synthetic target peptide:
   (i) is about or at least 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids long, optionally between 8 and 50 amino acids long; and
   (ii) comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 96, 206, 1-95, 97-205, 207-448, and 502-529, optionally wherein the synthetic target peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, 502, and 509-529,

   and further wherein said composition optionally has the ability to stimulate a T cell-mediated immune response to at least one of the synthetic target peptides and/or is capable of eliciting a memory T cell response to at least one of the synthetic target peptides.
2. The composition of claim 1, wherein at least one of the synthetic target peptides comprises a substitution of a serine residue with a homo-serine residue.
3. The composition of claims 1 or 2, wherein at least one of the synthetic target peptides is a phosphopeptide comprising phosphoserine, phosphothreonine, or phosphotyrosine.
4. The composition of any one of claims 1-3, wherein at least one of the synthetic target peptides comprises a phosphopeptide set forth in Tables 2-14.
5. The composition of claims 1 or 2, wherein at least one of the synthetic target peptides comprises a phosphopeptide mimetic comprising a mimetic of phosphoserine, phosphothreonine, or phosphotyrosine.

6. The composition of any one of claims 1, 2, or 5, wherein at least one of the synthetic target peptides comprises a phosphopeptide mimetic of a phosphopeptide set forth in Tables 2-14.
7. The composition of claim 6, wherein the phosphopeptide mimetic is resistant to dephosphorylation by a phosphatase enzyme.
8. The composition of claim 6, wherein the phosphopeptide mimetic is a synthetic molecule in which a phosphorous atom is linked to a serine, threonine, or tyrosine amino acid residue through a carbon.
9. The composition of claim 1, wherein the composition is immunologically suitable for use in a hepatocellular carcinoma (HCC) patient and/or an esophageal cancer patient.
10. The composition of claim 1, wherein the composition comprises at least 2, 3, 4, or 5 different target peptides.
11. The composition of claim 1, wherein the composition comprises at least 10 different target peptides.
12. The composition of claim 1, wherein the composition comprises at least 15 different target peptides.
13. The composition of claim 1, wherein at least one of the synthetic target peptides is capable of binding to an MHC class I molecule selected from the group consisting of an HLA-A*0201 molecule, an HLA A*0101 molecule, an HLA A*0301 molecule, an HLA B*4402 molecule, an HLA B*0702 molecule, and an HLA B*2705 molecule.
14. The composition of claim 1, wherein the composition is capable of increasing the 5-year survival rate of HCC patients and/or esophageal cancer patients treated with the composition by at least 20 percent relative to average 5-year survival rates that could have been expected without treatment with the composition.
15. The composition of claim 1, wherein the composition is capable of increasing the survival rate of HCC and/or esophageal cancer patients treated with the composition by at least 20 percent relative to a survival rate that could have been expected without treatment with the composition.
16. The composition of claim 1, wherein the composition is capable of increasing the treatment response rate of HCC and/or esophageal cancer patients treated with the

composition by at least 20 percent relative to a treatment rate that could have been expected without treatment with the composition.

17. The composition of claim 1, wherein the composition is capable of increasing the overall median survival of patients of HCC and/or esophageal cancer patients treated with the composition by at least two months relative to an overall median survival that could have been expected without treatment with the composition.

18. The composition of claim 1, further comprising at least one peptide derived from MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGE), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, β-HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein/cyclophilin C-associated protein), TAAL6, TAG72, TLP, and TPS.

19. The composition of claim 1, wherein the composition further comprises an adjuvant selected from the group consisting of montanide ISA-51, QS-21, a tetanus helper peptide, GM-CSF, cyclophosamide, bacillus Calmette-Guerin (BCG), corynbacterium parvum, levamisole, azimezone, isoprinisone, dinitrochlorobenezene (DNCB), keyhole limpet hemocyanin (KLH), complete Freunds adjuvant, in complete Freunds adjuvant, a mineral gel, aluminum hydroxide (Alum), lysolecithin, a pluronic polyol, a polyanion, an adjuvant peptide, an oil emulsion, dinitrophenol, and diphtheria toxin (DT), or any combination thereof.

20. An *in vitro* population of dendritic cells comprising the composition of any one of claims 1-19.

21. An *in vitro* population of $CD8^+$ T cells capable of being activated upon being brought into contact with a population of dendritic cells, wherein the dendritic cells comprise a composition of any one of claims 1-19.

22. An antibody or antibody-like molecule that specifically binds to a complex of an MHC class I molecule and a peptide, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 96, 206, 1-95, 97-205, 207-448, and 502-529, optionally an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, 502, and 509-529.

23. The antibody or antibody-like molecule of claim 22, wherein the peptide comprises a phosphopeptide set forth in Tables 2-14.

24. The antibody or antibody-like molecule of claim 22, wherein the phosphopeptide and corresponding MHC class I molecule are selected from Tables 2-14.

25. The antibody or antibody-like molecule of any one of claims 17-24, wherein the antibody or antibody-like molecule is a member of the immunoglobulin superfamily.

26. The antibody or antibody-like molecule of any one of claims 17-24, wherein the antibody or antibody-like molecule comprises a binding member selected from the group consisting an Fab, Fab', $F(ab')_2$, Fv, and a single-chain antibody.

27. The antibody or antibody-like molecule of any one of claims 17-24, conjugated to a therapeutic agent selected from the group consisting of an alkylating agent, an antimetabolite, a mitotic inhibitor, a taxoid, a vinca alkaloid, and an antibiotic.

28. The antibody or antibody-like molecule of any one of claims 17-24, wherein the antibody or antibody-like molecule is a T cell receptor, optionally conjugated to a CD3 agonist.

29. An *in vitro* population of T cells transfected with a nucleic acid, optionally an mRNA, encoding a T cell receptor of claim 28.

30. A method for treating and/or preventing cancer comprising administering to a subject in need thereof a therapeutically effective dose of a composition of any of claims 1-19 and/or a composition comprising at least one target peptide comprising an amino acid sequence as set forth in any of SEQ ID NOs: 96, 206, 1-95, 97-205,

207-448, and 502-529, optionally an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, 502, and 509-529.

31. The method of claim 30, wherein the cancer is HCC, and the at least one target peptide comprises an amino acid sequence as set forth in any of SEQ ID NOs: 96, 206, 1-95, 97-205, 207-448, and 509-529, optionally an amino acid sequence as set forth in any of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, and 509-529.

32. The method of claim 30, wherein the cancer is esophageal cancer, and the at least one target peptide comprises an amino acid sequence as set forth in any of SEQ ID NOs: 16, 36, 49, 54, 81, 105, 111, 137, 139, 140, 149, 156, 159, 166, 182, 191, 193, 196, 205, 216, 242, 249, 252, 257, 259, 262, 268, 269, 271, 289, 294, 296, 374, 376, 380, 381, 385, 428, and 502-508, optionally an amino acid sequence as set forth in any of SEQ ID NOs: 149, 385, and 502.

33. A method of treating and/or preventing hepatocellular carcinoma (HCC) and/or esophageal cancer comprising administering to a subject in need thereof a therapeutically effective dose of a composition of any of claims 1-19 or a composition comprising at least one target peptide in combination with a pharmaceutically acceptable carrier.

34. A method for treating and/or preventing cancer, optionally hepatocellular carcinoma (HCC) and/or esophageal cancer, comprising administering to a subject in need thereof a therapeutically effective dose of the $CD8^+$ T cells of claim 21 in combination with a pharmaceutically acceptable carrier.

35. A method for treating and/or preventing cancer, optionally hepatocellular carcinoma (HCC) and/or esophageal cancer, comprising administering to a subject in need thereof an *in vitro* population of dendritic cells of claim 20 in combination with a pharmaceutically acceptable carrier.

36. A method for treating and/or preventing hepatocellular carcinoma (HCC) and/or esophageal cancer, comprising administering to a subject in need thereof the population of $CD8^+$ T cells of claim 21 in combination with a pharmaceutically acceptable carrier.

37. A method for making a cancer vaccine, optionally a cancer vaccine for use in treating and/or preventing hepatocellular carcinoma (HCC) and/or esophageal cancer, comprising combining the composition of any of claims 1-19 with an the adjuvant selected from the group consisting of montanide ISA-51, QS-21, a tetanus helper peptide, GM-CSF, cyclophosamide, bacillus Calmette-Guerin (BCG), corynbacterium parvum, levamisole, azimezone, isoprinisone, dinitrochlorobenezene (DNCB), keyhole limpet hemocyanin (KLH), complete Freunds adjuvant, in complete Freunds adjuvant, a mineral gel, aluminum hydroxide (Alum), lysolecithin, a pluronic polyol, a polyanion, an adjuvant peptide, an oil emulsion, dinitrophenol, and diphtheria toxin (DT), or any combination thereof and a pharmaceutically acceptable carrier; and placing the composition, adjuvant, and pharmaceutical carrier into a container, optionally into a syringe.

38. A method for screening target peptides for inclusion in an immunotherapy composition of claims 1-19 or for use in the method of using a composition of claims 1-19, comprising:

(a) administering the target peptide to a human;

(b) determining whether the target peptide is capable of inducing a target peptide-specific memory T cell response in the human; and

(c) selecting the target peptide for inclusion in an immunotherapy composition if the target peptide elicits a memory T cell response in the human.

39. A method for determining a prognosis of a hepatocellular carcinoma (HCC) patient and/or an esophageal cancer patient, the method comprising:

(a) administering to the patient a target peptide comprising an amino acid sequence as set forth in any of SEQ ID NOs: 96, 206, 1-95, 97-205, 207-

448, and 502-529, optionally an amino acid sequence as set forth in any of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, and 509-529, wherein the target peptide is associated with the patient's HCC and/or esophageal cancer;

(b) determining whether the target peptide is capable of inducing a target peptide-specific memory T cell response in the patient; and

(c) determining that the patient has a better prognosis if the patient mounts a memory T cell response to the target peptide than if the patient did not mount a memory T cell response to the target peptide.

40. A kit comprising at least one target peptide composition comprising at least one target peptide comprising an amino acid sequence as set forth in any of SEQ ID NOs: 96, 206, 1-95, 97-205, 207-448, and 502-529, optionally an amino acid sequence as set forth in any of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, and 509-529, and a cytokine and/or an adjuvant.

41. The kit of claim 40, comprising at least 2, 3, 4, or 5 target peptide compositions.

42. The kit of claim 40, wherein the at least one target peptide composition is one of the compositions of claims 1-19.

43. The kit of claim 40, wherein the cytokine is selected from the group consisting of a transforming growth factor (TGF), optionally TGF-alpha and/or TGF-beta; insulin-like growth factor-I; insulin-like growth factor-II; erythropoietin (EPO); an osteoinductive factor; an interferon, optionally interferon-alpha, interferon-beta,

and/or interferon-gamma; and a colony stimulating factor (CSF), optionally macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), and/or granulocyte-CSF (G-CSF).

44. The kit of claim 40, wherein the adjuvant is selected from the group consisting of montanide ISA-51, QS-21, a tetanus helper peptide, GM-CSF, cyclophosphamide, bacillus Calmette-Guerin (BCG), corynbacterium parvum, levamisole, azimezone, isoprinisone, dinitrochlorobenezene (DNCB), a keyhole limpet hemocyanin (KLH), complete Freund's adjuvant, incomplete Freund's adjuvant , a mineral gel, aluminum hydroxide, lysolecithin, a pluronic polyol, a polyanion, an adjuvant peptide, an oil emulsion, dinitrophenol, and diphtheria toxin (DT).

45. The kit of claim 40, wherein the cytokine is selected from the group consisting of a nerve growth factor, optionally nerve growth factor (NGF) beta; a platelet-growth factor; a transforming growth factor (TGF), optionally TGF-alpha and/or TGF-beta; insulin-like growth factor-I; insulin-like growth factor-II; erythropoietin (EPO); an osteoinductive factor; an interferon, optionally interferon-α, interferon-β, and/or interferon-γ; a colony stimulating factor (CSF), optionally macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), and/or granulocyte-CSF (G-CSF); an interleukin (IL), optionally IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-13, IL-14, IL-15, IL-16, IL-17, and/or IL-18; LIF; EPO; kit-ligand; fms-related tyrosine kinase 3 (FLT-3; also called CD135); angiostatin; thrombospondin; endostatin; tumor necrosis factor; and lymphotoxin (LT).

46. The kit of claim 40, further comprising at least one peptide derived from MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGE), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, β-HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50,

MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, and TPS.

47. The kit of claim 40, wherein the at least one target peptide comprises an amino acid sequence as set forth in any of SEQ ID NOs: 96, 140, 206, 296, 1-95, 97-139, 141-205, 207-295, 297-448, and 502-529, optionally an amino acid sequence as set forth in any of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 226, 231-233, 237, 243, 245, 253, 261, 266, 270, 274, 275, 276, 281, 285-287, 292, 293, 295, 297, 299, 303-305, 317, 320, 337, 338, 340, 343-349, 351-364, 367-371, 373, 377, 379, 382, 383, 385, 386, 393-412, 414-426, 429-436, 438-448, 464, and 509-529.

48. The kit of any one of claims 40-47, wherein the at least one target peptide is selected from the group consisting of SEQ ID NOs: 96, 206, 1-95, 97-205, 207-224, 502-508, 515-520, 524, 525, 527, and 528, and any combination thereof.

49. The kit of any one of claims 40-48, wherein the at least one target peptide composition comprises one or more synthetic target peptides that specifically bind to an HLA molecule listed in Table 1 and/or that comprises an amino acid sequence at least 90% identical, optionally 100% identical, to one of the SEQ ID NOs: listed in Tables 2, 3, 5-7, and 14.

50. The kit of any one of claims 40-49, wherein the kit comprises at least two synthetic target peptides, wherein the at least two synthetic target peptides are in separate containers.

51. The kit of any one of claims 40-50, further comprising instructions related to determining whether the at least one synthetic target peptide of the at least one synthetic target peptide composition is capable of inducing a T cell memory response that is a T cell central memory response (Tcm) when the at least one synthetic target peptide composition is administered to a patient.

52. The kit of any one of claims 40-51, wherein the kit further comprises a tetanus peptide.

53. The kit of claim 52, wherein the tetanus peptide comprises an amino acid sequence that is at least 90%, 95%, or 100% identical to SEQ ID NO: 449 or SEQ ID NO: 450.

54. The kit of claim 52 or claim 53, wherein the tetanus peptide is about or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 natural or non-natural amino acids in length.

55. The kit of any one of claims 52-54, wherein the tetanus peptide comprises an amino acid sequence that is at least 90% identical to a 10-25 amino acid subsequence of a wild type tetanus toxoid protein.

56. The kit of any one of claims 52-55, wherein the tetanus peptide binds to one or more MHC Class II molecules when administered to a subject.

57. The composition of any one of claims 1-19, comprising a peptide capable of binding to an MHC class I molecule selected from the group consisting of an HLA-A*0201 molecule, an HLA A*0101 molecule, an HLA A*0301 molecule, an HLA B*4402 molecule, an HLA B*0702 molecule, and an HLA B*2705 molecule.

58. The composition of any one of claims 1-19 and 50, wherein at least one of the synthetic peptides comprises an amino acid sequence selected from the group consisting of 96, 206, 1-95, 97-205, 207-224, 502-508, 515-520, 524, 525, 527, and 528, optionally an amino acid sequence as set forth in any of SEQ ID NOs: 4, 5, 10, 11, 15, 24, 32, 33, 37, 38, 41, 42, 52, 59, 63, 64, 66, 72, 75, 80, 83-89, 91, 95, 96, 106-108, 113, 115-117, 122, 123, 127, 128, 130-132, 146-149, 157, 158, 160, 161, 163-165, 167, 174, 179, 181, 185-188, 195, 198, 203, 206, 210, 212, 215, 218, 221, 222, 224, 502, 515-520, 524, 525, 527, and 528.

59. The composition of any one of claims 1-19 and 58, wherein the composition further comprises a tetanus peptide.

60. The composition of claim 59, wherein the tetanus peptide comprises an amino acid sequence that is at least 90%, 95%, or 100% identical to SEQ ID NO: 449 or SEQ ID NO: 450.

61. The composition of any one of claims 59 and 60, wherein the tetanus peptide is about or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 natural or non-natural amino acids in length.

62. The composition of any one of claims 59-61, wherein the tetanus peptide comprises an amino acid sequence that is at least 90% identical to a 10-25 amino acid subsequence of a wild type tetanus toxoid protein.

63. The composition of any one of claims 59-62, wherein the tetanus peptide binds to one or more MHC Class II molecules when administered to a subject.

64. The composition of any one of claims 59-63, wherein the tetanus peptide is modified so as to prevent formation of tetanus peptide secondary structures.

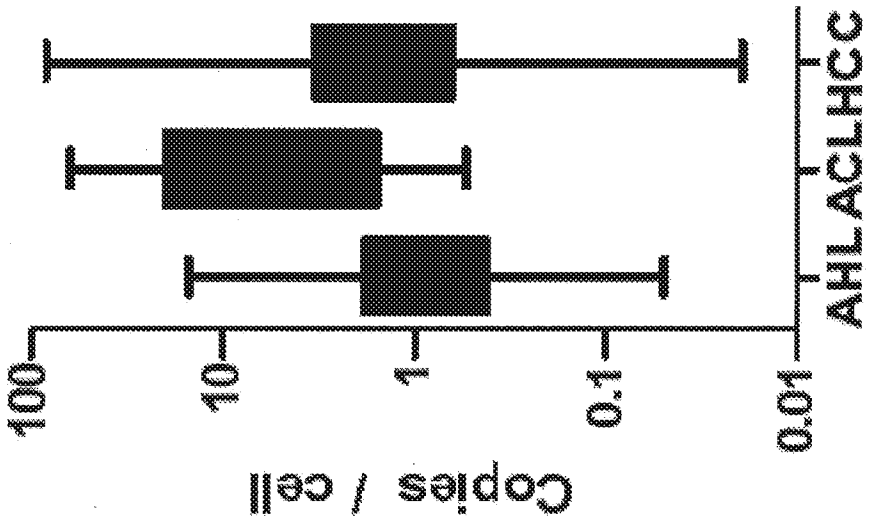
Copies / cell
100
10
1
0.1
0.01
AHL
ACL
HCC

FIG. 1B

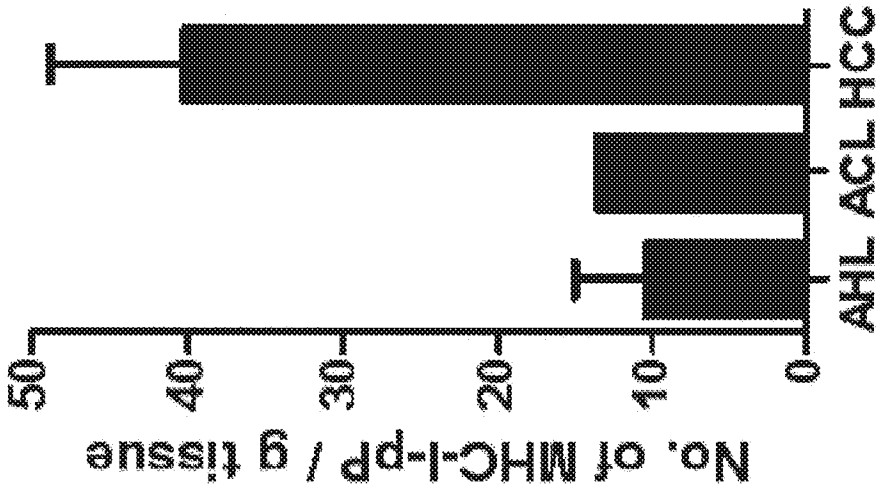
No. of MHC-I-pP / g tissue
50
40
30
20
10
0
AHL
ACL
HCC

FIG. 1A

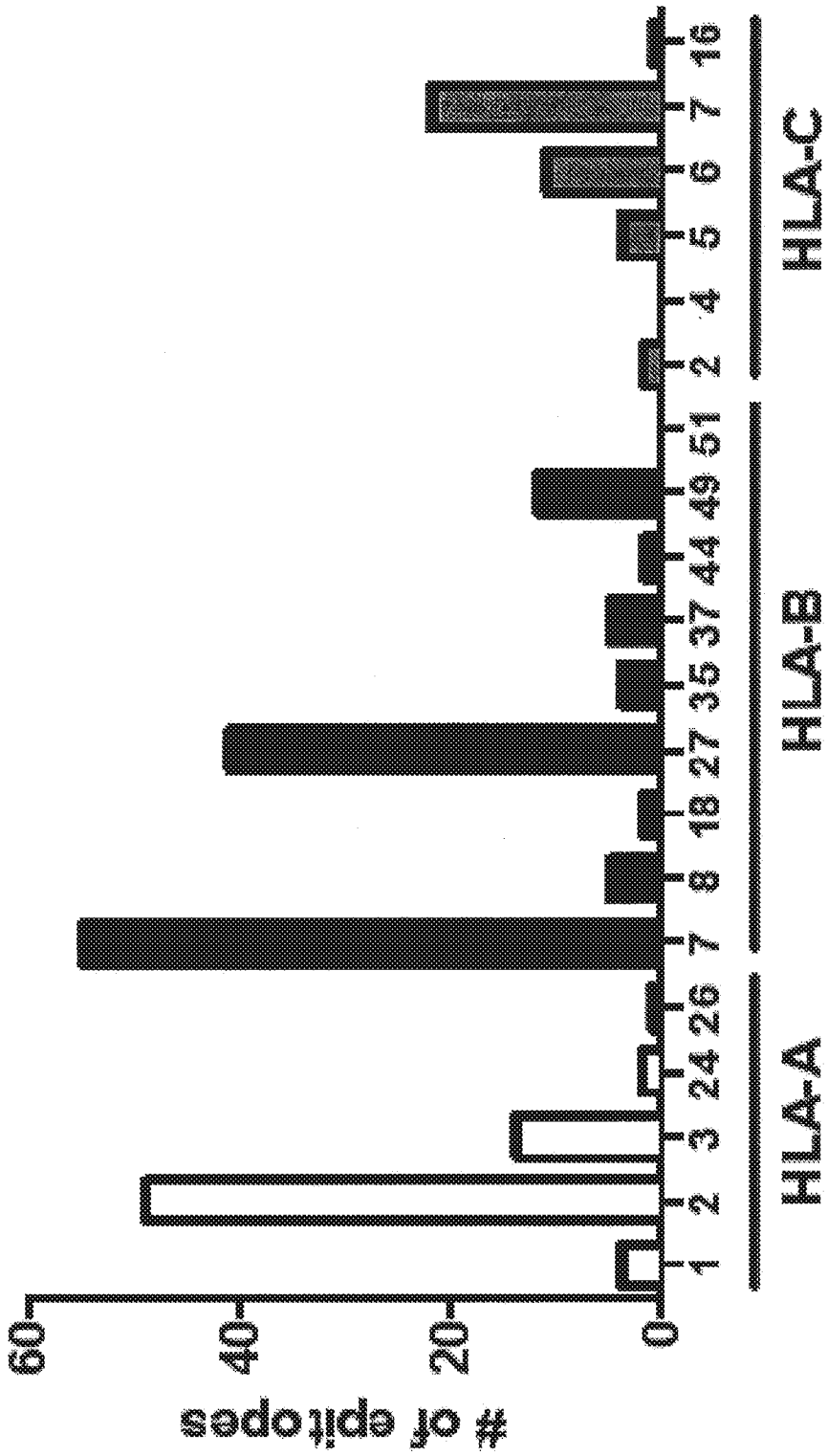
# of epitopes
60
40
20
0
1
2
3
24
26
7
8
18
27
35
37
44
49
51
2
4
5
6
7
16
HLA-A
HLA-B
HLA-C

FIG. 1C

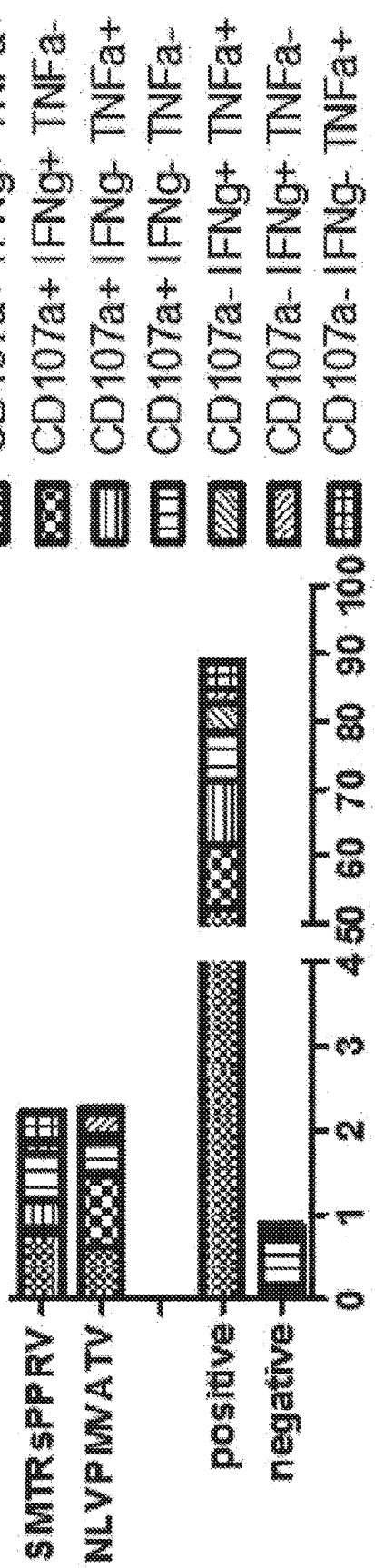
CD107a+ IFNg+ TNFa+
CD107a+ IFNg+ TNFa-
CD107a+ IFNg- TNFa+
CD107a+ IFNg- TNFa-
CD107a- IFNg+ TNFa+
CD107a- IFNg+ TNFa-
CD107a- IFNg- TNFa+
SMTRsPPRV
NLVPMWATV
positive
negative
0 1 2 3 4 50 60 70 80 90 100
% cytokine production

FIG. 2

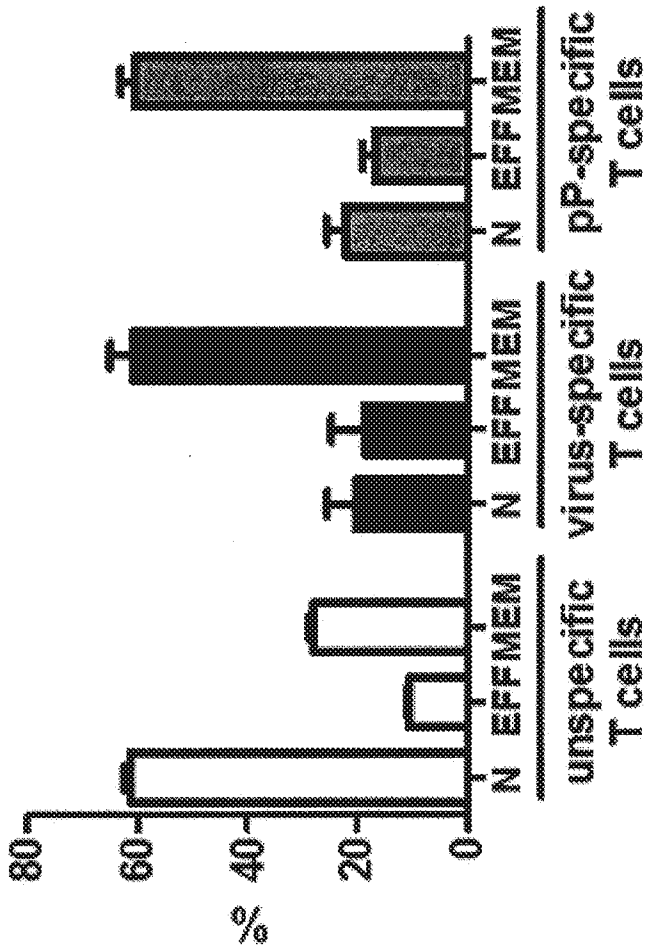
80
60
40
20
0
%
N EFFMEM N EFFMEM
unspecific T cells
virus-specific T cells
pP-specific T cells

FIG. 3B

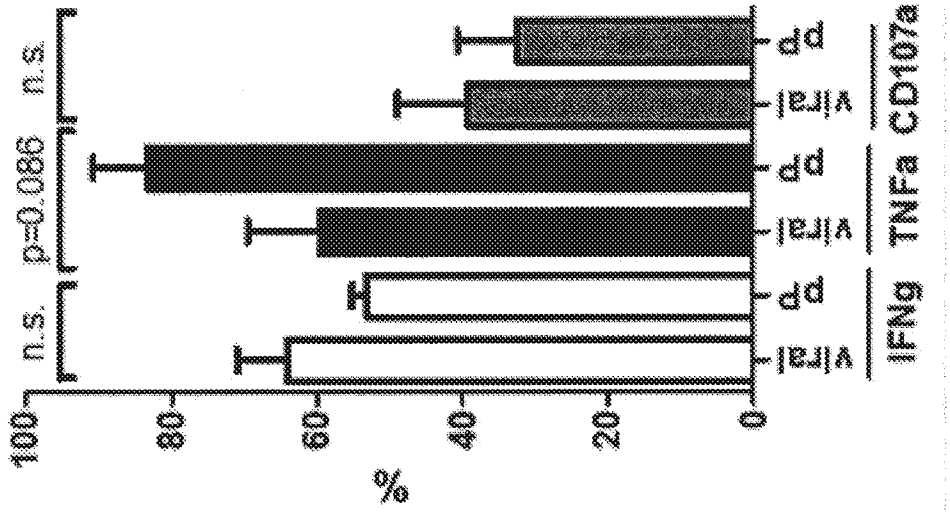
100
80
60
40
20
0
%
n.s.
p=0.086
n.s.
vTal
pp
vTal
pp
vTal
pp
IFNg
TNFa
CD107a

FIG. 3A

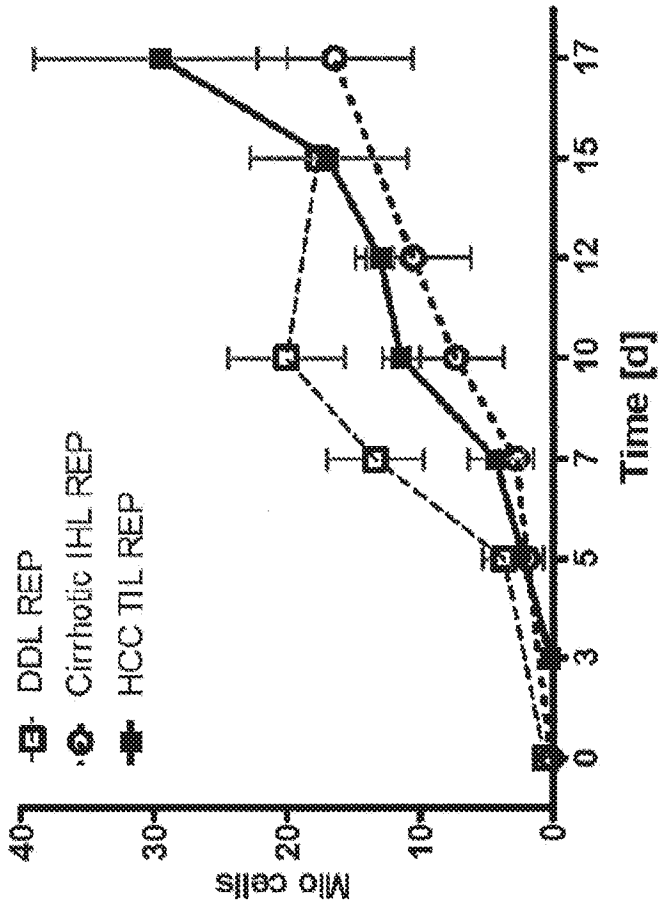
DDL REP
Cirrhotic IHL REP
HCC TIL REP
Mio cells
Time [d]
0
10
20
30
40
0
3
5
7
10
12
15
17

FIG. 4A

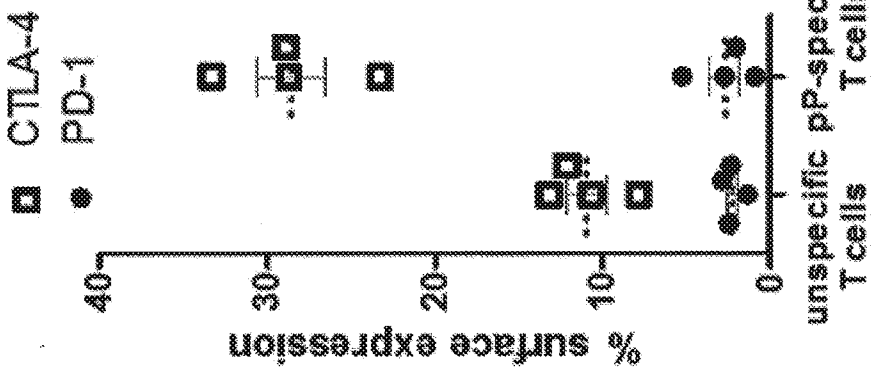
CTLA-4
PD-1
% surface expression
0
10
20
30
40
unspecific T cells
pP-specific T cells

FIG. 3C

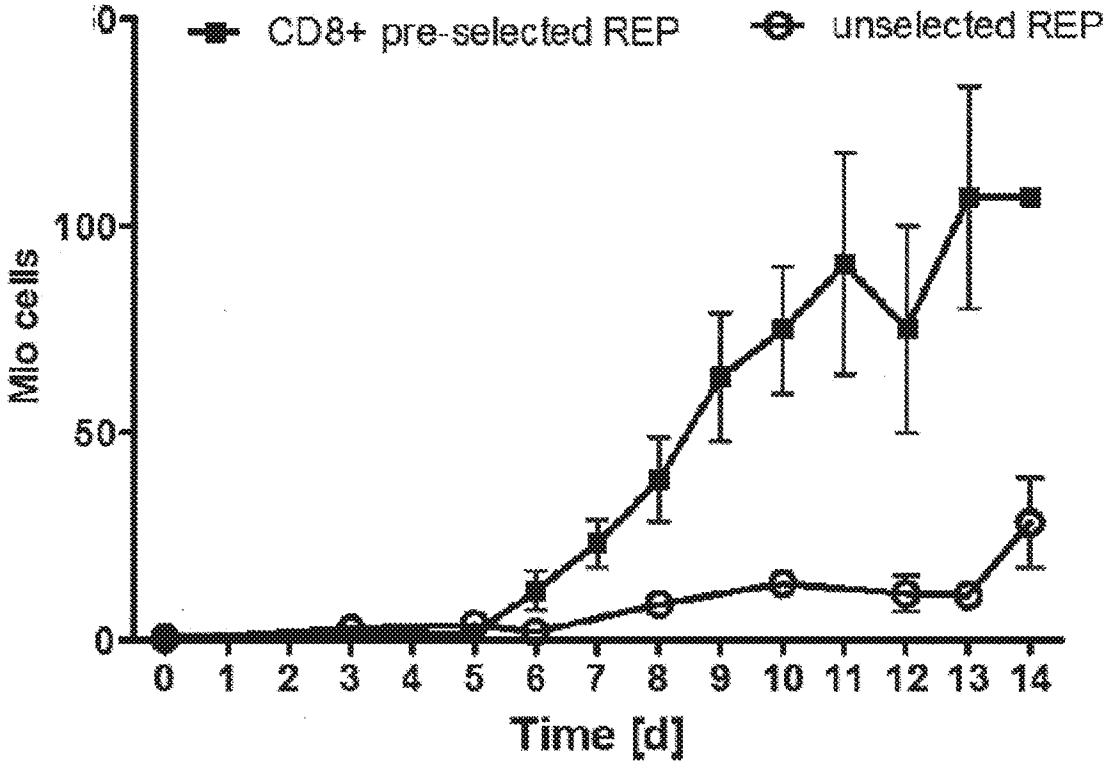
CD8+ pre-selected REP
unselected REP
Mio cells
0
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time [d]

FIG. 4B

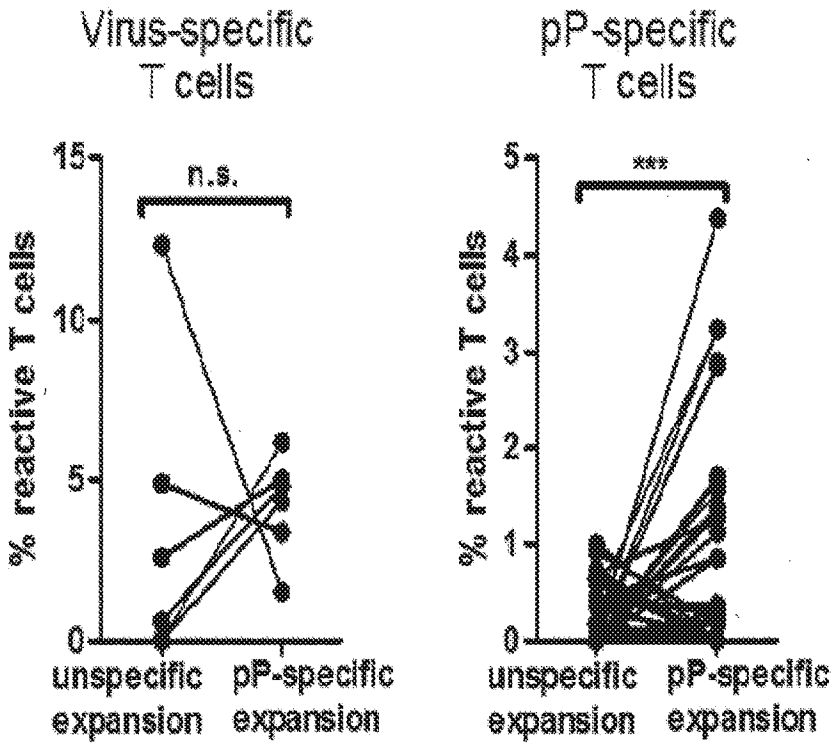
Virus-specific T cells
pP-specific T cells
n.s.
***
% reactive T cells
15
10
5
0
5
4
3
2
1
0
unspecific expansion
pP-specific expansion
unspecific expansion
pP-specific expansion

FIG. 5A

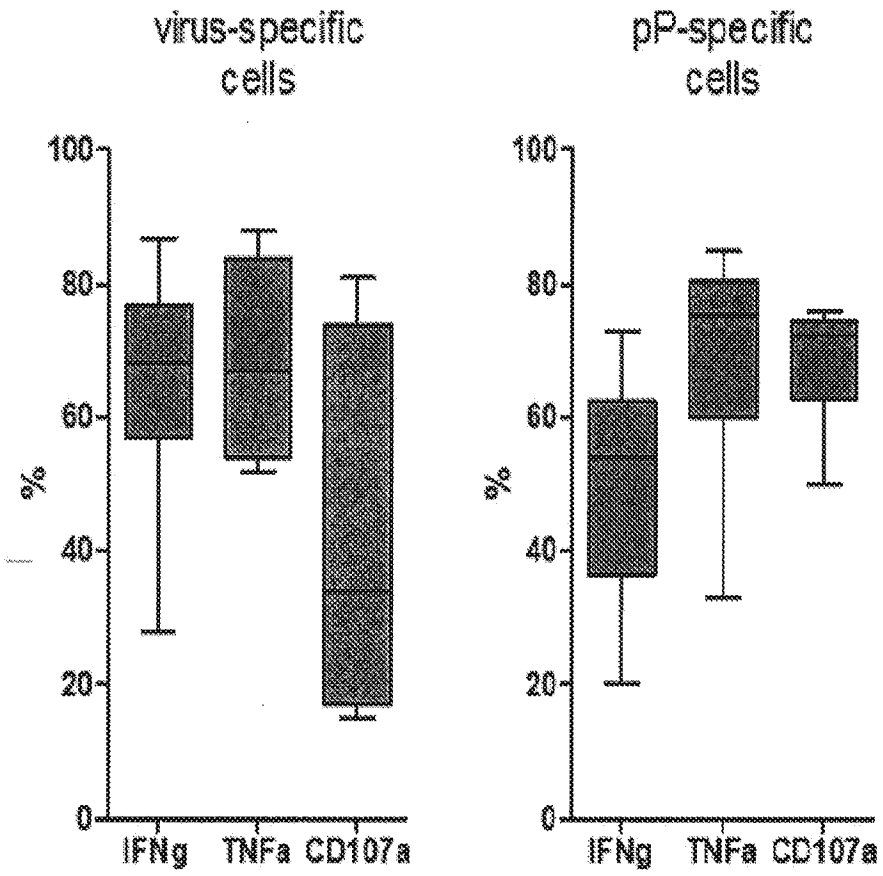
virus-specific cells
pP-specific cells
100
80
60
40
20
0
%
IFNg
TNFa
CD107a

FIG. 5B

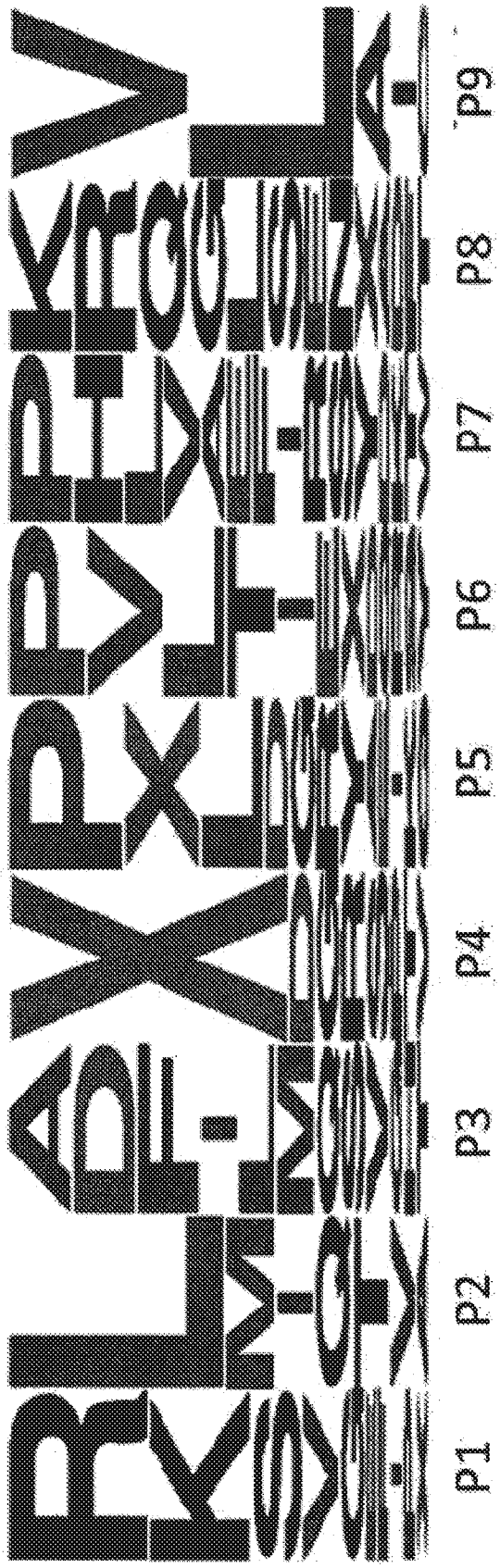

FIG. 6

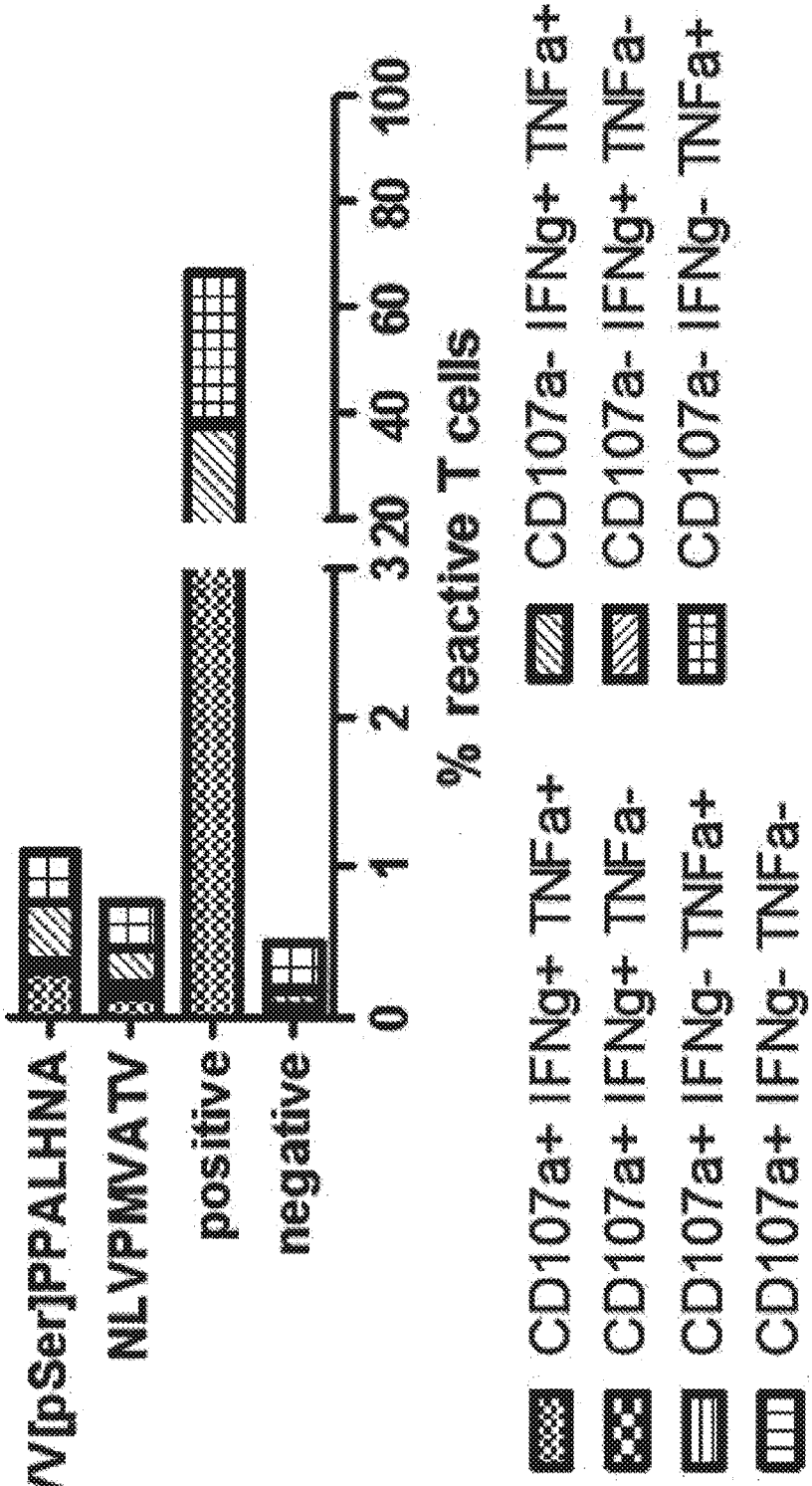
AW[pSer]PPALHNA
NLVPMVATV
positive
negative
0
1
2
320
40
60
80
100
% reactive T cells
CD107a+ IFNg+ TNFa+
CD107a+ IFNg+ TNFa-
CD107a+ IFNg- TNFa+
CD107a+ IFNg- TNFa-
CD107a- IFNg+ TNFa+
CD107a- IFNg+ TNFa-
CD107a- IFNg- TNFa+
CD107a- IFNg- TNFa-

FIG. 7

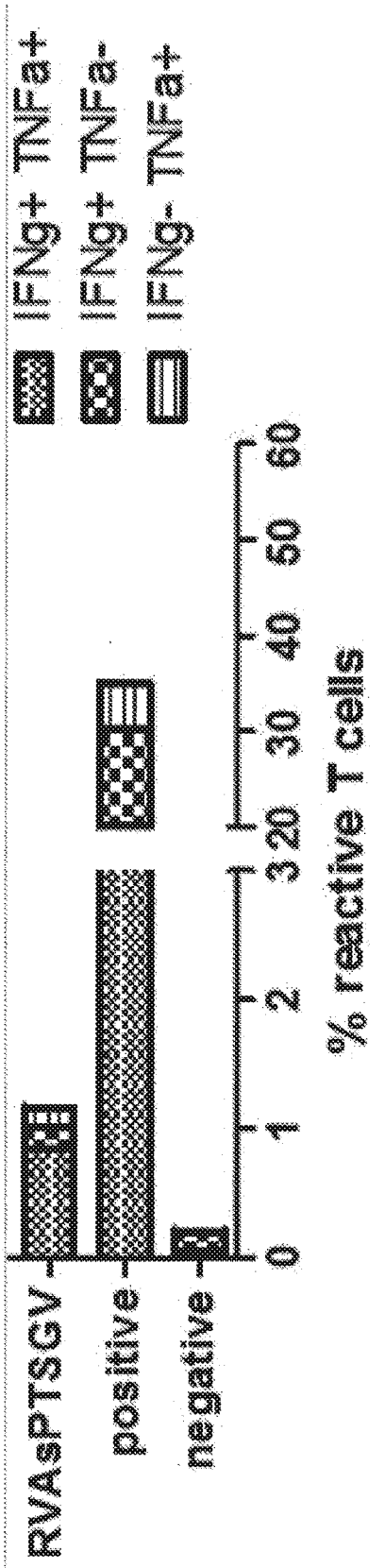
IFNg+ TNFa+
IFNg+ TNFa-
IFNg- TNFa+
RVAsPTSGV
positive
negative
0
1
2
3
20
30
40
50
60
% reactive T cells

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2017/031266

A. CLASSIFICATION OF SUBJECT MATTER

(see extra sheet)

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)

A61K 39/00, 39/395, C07K 7/04, 7/06, A61P 35/00, C12N 5/07, 5/0783, 5/0784.

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)

PatSearch, EMBL, NCBI, PAJ, Espacenet, DWPI, PCT Online, USPTO DP, CIPO (Canada PO), SIPO DB, Freepatentsonline, FASTA, NucleotideBLAST.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/039675 A2 (UNIVERSITY OF VIRGINIA PATENT FOUND et al.) 13.03.2014, abstract, p.6-13, 19-34, 36, 39-40, 49-50	1-3, 5, 9-49
A	WO 2011/149909 A2 (HUNT DONALD F. et al.) 01.12.2011, abstract, claims	1-3, 5, 9-49
A	PAGES FRANCK et al. In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. Journal of clinical oncology, 2009, Vol.27, no.35, pp.5944-5951	1-3, 5, 9-49

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 July 2017 (10.07.2017)	24 August 2017 (24.08.2017)
Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhkovskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37	Authorized officer U.Berezhnaya Telephone No. 495 531 65 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2017/031266

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4, 6-8, 50-64
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Remark on Protest

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

A61K 39/00 *(2006.01)*
A61K 39/395 *(2006.01)*
C07K 7/06 *(2006.01)*
C07K 7/04 *(2006.01)*
A61P 35/00 *(2006.01)*
C12N 5/0783 *(2006.01)*
C12N 5/0784 *(2006.01)*