(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 December 2011 (15.12.2011)

PCT

(10) International Publication Number
WO 2011/154863 A1

(51) International Patent Classification:
C07K 14/71 (2006.01) *A61K 39/00* (2006.01)
C07K 14/82 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/IB2011/052246

(22) International Filing Date:
23 May 2011 (23.05.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/352,318 7 June 2010 (07.06.2010) US

(71) Applicant *(for all designated States except US)*: **PFIZER INC.** [US/US]; 235 East 42nd Street, New York, New York 10017 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CHO, Helen Kim** [KR/US]; Pfizer Global Research & Development, 10777 Science Center Drive, San Diego, California 92121 (US). **GURU, Siradanahalli Chandrasekharaiah** [IN/US]; Pfizer Global Research & Development, 10777 Science Center Drive, San Diego, California 92121 (US). **TSAI, Van To** [US/US]; Pfizer Global Research & Development, 10777 Science Center Drive, San Diego, California 92121 (US). **PIERCE, Brian Gregory** [US/US]; University of Massachusetts Medical School, 364 Plantation St., LRB 1004, Worcester, MA 01605-4321 (US).

(74) Agents: **KLEIMAN, Gabriel L.** et al.; Pfizer Inc., 235 East 42nd Street, New York, NY 10017 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

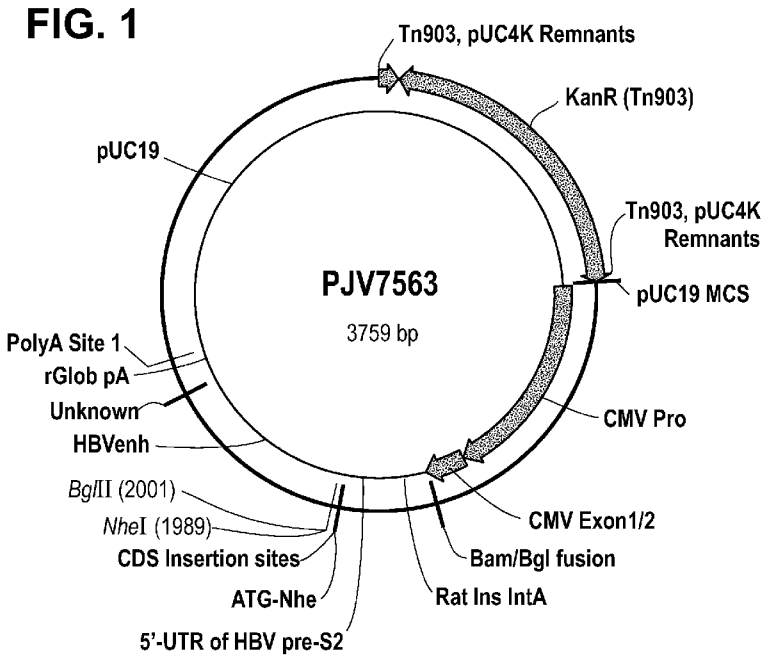
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

[Continued on next page]

(54) Title: HER-2 PEPTIDES AND VACCINES

FIG. 1



(57) Abstract: The present invention provides (a) isolated immunogenic HER-2 peptides capable of inducing immune responses against human HER-2 receptor; (b) isolated nucleic acid molecules encoding an isolated immunogenic HER-2 peptide; (c) plasmid constructs comprising a nucleic acid molecule encoding an isolated immunogenic HER- 2 peptide; (d) vaccine compositions comprising an isolated immunogenic HER-2 peptide (e) vaccine compositions comprising an isolated nucleic acid molecule encoding an isolated immunogenic HER-2 peptide; and (f) methods of treating or preventing cancer, inhibiting abnormal cell proliferation, or eliciting an immune response against HER-2 protein in a mammal using (1) an isolated immunogenic HER-2 peptide, (2) nucleic acid molecule encoding an isolated immunogenic HER-2 peptide, or (3) a composition comprising an isolated immunogenic HER-2 peptide, or composition comprising a nucleic acid molecule encoding an isolated immunogenic HER-2 peptide.

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— *as to the identity of the inventor (Rule 4.17(i))*

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

HER-2 PEPTIDES AND VACCINES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 61/352,318 filed on June 7, 2010, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to vaccines useful for treating or preventing cancer, and specifically to vaccines against cancer disease that is associated with HER-2.

BACKGROUND OF THE INVENTION

Tumor antigens are a group of proteins expressed by tumor cells. These antigens are divided into five categories according to their expression profile: (1) antigens specific for the patient, resulting from point mutations related to tumorigenesis, (2) tumor-specific antigens (TSA) expressed in many tumors and a few normal tissues devoid of conventional HLA molecules, (3) differentiation antigens which are expressed either during embryogenesis or in quite specific cell types, (4) antigens over expressed by tumors (such as survivin, gp75, PSA, HER-2, p53, and telomerase) and (5) viral antigens.

Human epidermal growth factor receptor-2 (also known as HER-2, HER-2/neu, c-erbB-2 or p185; hereinafter referred to as HER-2, HER-2 receptor, or HER-2 protein) is a 185 kDa protein that belongs to the epidermal growth factor receptor family. Sequences of human HER-2 and its orthologs are available from the NCBI web site: http://www.ncbi.nlm.nih.gov/, where the sequences are identified with the following RefSeq Identifiers: NP_004439.2 (human), XP_001090319.1 (rhesus), XP_001501155.1 (horse), NP_001003217.1 (dog), NP_001041628.1 (cat), NP_058699.2 (rat), and NP_001003817.1 (mouse). The amino acid sequence of the full length human HER-2 protein is provided in SEQ ID NO: 1. The DNA sequence encoding the amino acid sequence of the full length human HER-2 protein is provided in SEQ ID NO: 2. Human HER-2 protein consists of an extracellular domain (ECD) (amino acids 1-653), a transmembrane domain (TMD)(amino acids 654-675), and an intracellular domain (ICD)(amino acids 676-1255). The ECD includes a signal sequence that consists of amino acids 1 - 22. The ICD includes a tyrosine kinase domain (amino acids 720-787) and a carboxy terminal (C-terminal) domain (CTD)(amino acids 991-1255).

HER-2 protein has been found to be amplified and over expressed in several types of human adenocarcinomas, especially in tumors of the breast and the ovary. For

example, HER-2 was found to be over expressed (3+) in 15-25% and moderately (1+) to highly (2+) expressed in 30-45% of the breast cancer patients. (See Perez EA, et al, HER2 testing in patients with Breast Cancer: Poor correlation between weak positivity by immunohistochemistry and gene amplification by fluorescence in situ hybridization. Mayo Clin. Proc. 2002;77:148-154.) Therefore, for the purposes of this invention, such cancers are considered to be "cancers associated with HER-2."

Several vaccine strategies targeting tumors that over express HER-2 using peptides, proteins, plasmid DNA, and viral vector approaches have been explored. For example, US Patent 7,348,018 mentions a peptide of the HER-2 ECD domain and its use for eliciting or enhancing an immune response. Published US patent application US2006/074038 refers to a HER-2 expressing plasmid construct encoding a truncated HER-2 gene that lacked the intracellular domain and the use of such a construct as a vaccine. Many of these strategies have seen little or no clinical responses mainly due to use of a single antigen and/or self antigens to induce immune responses that do not break immune tolerance or adequately activate dendritic cells (DCs) and expand cytotoxic T lymphocytes (CTLs).

SUMMARY OF THE INVENTION

In one aspect, the present disclosure provides an isolated immunogenic HER-2 peptide capable of eliciting an immune response against the human HER-2 receptor, which comprises an amino acid sequence derived from the ECD of the human HER-2 protein ("ECD-derived peptide"). In some embodiments, the ECD-derived peptide contains the amino acid sequences of at least four of the conserved T cell epitopes in the ECD domain of human HER-2 receptor and shares from 70% to 95% identity with the ECD domain of human HER-2 receptor. In another aspect, the present disclosure provides an isolated immunogenic HER-2 peptide capable of eliciting an immune response against human HER-2 receptor, which comprises an ECD-derived peptide linked to a CTD-derived peptide, wherein the ECD-derived peptide contains the amino acid sequences of at least four of the conserved T cell epitopes in the ECD domain of human HER-2 receptor and shares from 70% to 95% identity with the ECD domain of human HER-2 receptor, and wherein the CTD-derived peptide contains the amino acid sequence of the conserved T cell epitope in the CTD domain of human HER-2 protein and shares from 70% to 95% identity with the amino acid sequence of the CTD domain of the human HER-2 protein.

In another aspect, the present disclosure provides an isolated immunogenic HER-2 peptide capable of eliciting an immune response against human HER-2 receptor, which comprises an ECD-derived peptide, a CTD-derived peptide, and a TMD-derived peptide, wherein the carboxy terminus of the ECD-derived peptide is joined to the amino terminus of the TMD-derived peptide and the carboxy terminus of the TMD-derived peptide is joined to the amino terminus of the CTD-derived peptide. Preferably, the ECD-derived peptide, TMD-derived peptide, and CTD-derived peptide are each joined together by a peptide bond to form a fusion protein.

In another aspect, the present disclosure provides an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an isolated immunogenic HER-2 peptide provided by the present disclosure.

In another aspect, the present disclosure provides a composition, which comprises an isolated immunogenic HER-2 peptide or an isolated nucleic acid molecule, provided by the present disclosure, and a pharmaceutically acceptable excipient. In some embodiments, composition is a vaccine composition.

In yet another aspect, the present disclosure provides a method of inhibiting abnormal cell proliferation, eliciting an immune response against HER-2 protein, or treating or preventing cancer in a mammal, comprising administering to the mammal an effective amount of a vaccine composition provided by the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents a vector map of a plasmid for cloning RaDHER2. RaDHER2 and control genes (human and rat HER2) were cloned into PJV7563 at NheI (5') and BglII (3') sites.

Figure 2 shows results of a representative IFNγ ELISPOT assay to evaluate the ability of the RaDHER2 antigen to induce HER-2 specific CD8 T cells. The responses from two independent cultures showed specificity to HER2 p369 sequences (Figure 2a) and p435 peptide sequence (Figure 2b), respectively when compared to other HER-2 peptides tested.

Figure 3 shows results from a representative assay to further characterize the ability of the p435-specific-CD8 T cells induced by the RaDHER2 antigen to recognize tumor cells expressing native HER-2 in an IFNγ ELISPOT assay. Plot is normalized to the number of cells that secrete IFNγ in 1e6 CD8 cells.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions of Terms

The term “adjuvant” refers to a substance that is capable of enhancing, accelerating, or prolonging an immune response when given with a vaccine antigen.

The term "antigen" refers to a substance that is capable of inducing a specific humoral and/or cell-mediated immune response. An antigen generally contains at least one epitope. Examples of antigens include molecules which contain a peptide, polysaccharide, nucleic acid sequence, and/or lipid.

The term “immunogenic HER2 peptide” refers to a peptide that, when administered with an appropriate carrier and/or adjuvant, is capable of eliciting an immune response against human HER-2 protein or against cells expressing human HER-2 protein.

The term “conserved T cell epitope” refers to one of the following amino acid sequences of the human HER-2 protein as set forth in SEQ ID NO. 1: amino acids 5-16 (ALCRWGLLLALL) (HLA-A2/ HLA-A24), amino acids 48-56 (HLYQGCQVV) (HLA-A2), amino acids 98-114 (RLRIVRGTQLFEDNYAL) (HLA-DR/HLA-A2), amino acids 328-345 (TQRCEKCSKPCARVCYGL) (HLA-DR), amino acids 369-386 (KIFGSLAFLPESFDGDPA) (HLA-A2, -A3, -A26/HLA-DR), and amino acids 1023-1031 (YLVPQQGFF) (HLA-A2).

The term “CTD-derived peptide” refers to a peptide that comprises the amino acid sequence of the conserved T cell epitope (i.e., amino acids 1023-1031 of SEQ ID No.: 1) on the CTD of the human HER-2 protein and is at least 70% identical to the amino acid sequence of the CTD of the human HER-2 protein.

The term “ECD-derived peptide” refers to a peptide that comprises the amino acid sequences of at least four of the conserved T cell epitopes of the ECD of the human HER-2 and shares from 70% to 95% identity with the amino acid sequence of the ECD of the human HER-2 protein.

The term “effective amount” refers to an amount delivered to the subject that is sufficient to cause a desired effect in the subject.

The term "eliciting an immune response" means stimulating, initiating, or inducing an immune response, and/or improving, amplifying, enhancing, increasing or prolonging a pre-existing immune response.

The term “functional variant” of an immunogenic HER-2 peptide refers to a peptide that is at least 70% identical to the amino acid sequence of that immunogenic HER-2 peptide and is capable of inducing substantially the same immune response as that immunogenic HER-2 peptide.

The term "immune response" refers to any response to an antigen by the immune system of a vertebrate animal *in vivo*, or by one or more components of the immune system of a vertebrate animal *in vitro*. Exemplary immune responses include, but are not limited to, local and systemic cellular as well as humoral immunity, such as cytotoxic T lymphocytes (CTL) responses, including antigen-specific induction of $CD8^+$ CTLs, helper T-cell responses including T-cell proliferative responses and cytokine release, and B-cell responses including antibody response.

The term “pharmaceutically acceptable excipient” refers to a substance, other than the active ingredient or adjuvant, that is compatible with the active ingredient and does not cause significant untoward effect in subjects to whom it is administered.

The term "polypeptide," "peptide," and "protein," are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones.

The term “ortholog” refers to genes in different species that are similar to each other and originated from a common ancestor.

The term “preventing” or “prevent” refers to a) keeping a disorder from occurring or b) delaying the onset of a disorder or onset of a symptoms of a disorder.

The term “TMD-derived peptide” refers to a peptide that is at least 50% identical to the amino acid sequence of the transmembrane domain (TMD) of human HER-2 protein. A TMD-derived peptide may have amino acid sequence only from the CTD of human HER-2; it may also contain amino acid sequence from the cytoplasmic terminal domain (CTD) of human HER-2.

The term “treating,” “treatment,” or “treat” refers to abrogating a disorder, reducing the severity of a disorder, or reducing the severity or occurrence frequency of a symptom of a disorder.

B. Immunogenic HER-2 Peptides

In some aspects, the present disclosure provides isolated immunogenic HER-2 peptides capable of eliciting an immune response against human HER-2 protein or against cells expressing human HER-2 protein. The peptides comprise the amino acid sequences of at least four of the conserved T cell epitopes of human HER-2 protein and share from 70% to 95% identity with the amino acid sequence of human HER-2 protein. In some embodiments, the peptides comprise the amino acid sequences of at least five of the conserved T cell epitopes of human HER-2 and share from 70% to 95% identity,

from 75% to 85% identity, or from 85% to 95% identity, with the amino acid sequence of human HER-2 protein.

An immunogenic HER2 peptide of the invention may be derived by conserving some or all of the conserved T cell epitopes of the human HER-2 protein while substituting certain amino acids in the remaining regions of the human HER-2 protein with amino acids found in one or more orthologs of human HER-2 protein at corresponding positions. Examples of orthologs of human HER-2 include the HER-2 protein of rhesus monkey (XP_001090319.1), horse (XP_001501155.1), dog (NP_001003217.1), cat (NP_001041628.1), rat (NP_058699.2), mouse (NP_001003817.1), chimp (XR_025186.1), cow (XR_083057.1), and hamster (D16295.1). Substitutions of amino acids of human HER2 protein with amino acids from one or more of the orthologs may be conservative substitutions or non-conservative substitutions, or both, and may be selected based on a number of factors known in the art, including the divergence needed to be achieved, MHC binding, the presence of ortholog amino acids at the site of substitution, surface exposure, and maintaining the 3-D structure of the protein for optimal processing and presentation.

The capability of an isolated immunogenic HER-2 peptide to elicit an immune response can be measured in *in vitro* assays or *in vivo* assays. In one particular embodiment, the immune response is a T cell response measured in an *in vitro* assay. *In vitro* assays or tests for determining the capability of a peptide or DNA construct to elicit immune responses are known in the art. One example of such *in vitro* assays that may be used to determine the capability of an isolated polypeptide of the invention to elicit immune response is to measure the capability of the antigen to stimulate T cell response as described in US Patent 7,387,882, the disclosure of which is incorporated in this application. The assay method comprises the steps of: (1) contacting antigen presenting cells in culture with an antigen thereby the antigen can be taken up and processed by the antigen presenting cells, producing one or more processed antigens; (2) contacting the antigen presenting cells with T cells under conditions sufficient for the T cells to respond to one or more of the processed antigens; (3) determining whether the T cells respond to one or more of the processed antigens. The T cells used may be $CD8^+$ T cells or $CD4^+$ T cells. T cell response may be determined by measuring the release of one of more of cytokines, such as interferon-gamma and interleukin-2, lysis of the antigen presenting cells (tumor cells), and production of antibodies by B cells. One specific exemplary assay is described in Example 3 provided in the present application.

In one aspect, the immunogenic HER-2 peptide provided by this disclosure comprises an ECD-derived peptide. In some embodiments, the amino acid sequence of the ECD-derived peptide comprises the amino acid sequences of at least four, preferably at least five, of the conserved T cell epitopes of the ECD of the human HER-2 protein and shares from 70% to 85% identity with the amino acid sequence of the ECD of human HER-2 protein. In other embodiments, the amino acid sequence of the ECD-derived peptide comprises the amino acid sequences of at least four, preferably at least five of the conserved T cell epitopes of the ECD of the human HER-2 protein and shares from 85% to 95% identity with the amino acid sequence of the ECD of human HER-2 protein.

In some particular embodiments, the ECD-derived peptide comprises the amino acid sequences of the five conserved T cell epitopes of the ECD of the human HER-2 and includes, in positions outside of the conserved T cell epitopes in the ECD domain of the human HER-2, from 50 to 130 amino acids that are found in one or more of the human HER-2 orthologs in corresponding positions and are different from the amino acids found in the ECD domain of human HER-2.

In some further embodiments, the ECD-derived peptide comprises the amino acid sequences of five conserved T cell epitopes of the ECD of the human HER-2 and includes, in positions outside of the conserved T cell epitopes in the ECD of the human HER-2, from 120 to 130 amino acids that are found in one or more of the human HER-2 orthologs in corresponding positions and are different from the amino acids found in the ECD of human HER-2.

In still some further embodiments, the ECD-derived peptide comprises the amino acid sequences of five conserved T cell epitopes of the ECD of the human HER-2 and includes, in positions outside of the conserved T cell epitopes in the ECD of the human HER-2, from 50 to 70 amino acid that are found in one or more of the human HER-2 orthologs in corresponding positions and are different from the amino acids found in the ECD of human HER-2.

In a particular embodiment, the amino acid sequence of the ECD-derived peptide comprises amino acids 23 – 645 of SEQ ID NO.: 5 or amino acids 25 - 647 of SEQ ID NO: 14.

In still another particular embodiment, the amino acid sequence of the ECD-derived peptide is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to amino acids 23 – 645 of SEQ ID NO. 5, or is at least 80%, at least 85%,

at least 90%, at least 95%, or at least 99% identical to amino acids 25 – 647 of SEQ ID NO: 14.

In still another particular embodiment, the present invention provides an isolated peptide that comprises an amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO: 6 or encoded by the nucleic acid sequence of SEQ ID NO:15.

In another aspect, the present invention provides an isolated immunogenic HER-2 peptide that comprises an ECD-derived peptide and a CTD-derived peptide, wherein the C-terminal end of the ECD-derived peptide is joined to the N-terminal end of the CTD-derived peptide. In some embodiments, the ECD-derived peptide is joined to the N-terminal end of the CTD-derived peptide by a peptide bond to form a fusion peptide. The ECD-derived peptide is as described herein. In some embodiments, the amino acid sequence of the ECD-derived peptide comprises amino acids 23 – 645 SEQ ID NO: 5 or amino acids 25 – 647 of SEQ ID NO: 14

A CTD-derived peptide may be obtained by conserving the conserved T cell epitope (i.e., amino acids 1023-1031 of SEQ ID No.: 1) in the CTD of the human HER-2 protein while substituting certain amino acids in the remaining regions of the CTD of the human HER-2 protein with amino acids found in one or more orthologs of human HER-2 protein in corresponding positions. In one embodiment, the CTD-derived peptide shares from 70% to 95% identity with the amino acid sequence of CTD of the human HER-2. In another embodiment, the CTD-derived peptide contains from 15 to 70 amino acids that are found in corresponding positions in the CTD domain of one or more orthologs of the human HER-2 and are different from the amino acids in the CTD of human HER-2 protein. In a particular embodiment, the CTD-derived peptide has an amino acid sequence of SEQ ID NO: 7. In another particular embodiment, the CTD-derived peptide has an amino acid sequence of SEQ ID NO: 22.

In other particular embodiments, the CTD-derived peptide is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of SEQ ID NO. 7, or is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 22.

In a particular embodiment, the isolated immunogenic HER-2 peptide has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to amino acids 23 – 910 of SEQ ID NO: 3, or is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to amino acids 25 – 912 of SEQ ID NO: 18.

In a specific embodiment, the isolated immunogenic HER-2 peptide comprises amino acids 23 – 910 of SEQ ID NO: 3. In another specific embodiment, the isolated immunogenic HER-2 peptide comprises amino acids 25 – 912 of SEQ ID NO: 18. In another specific embodiment, the isolated immunogenic HER-2 peptide consists of amino acids 23 – 910 of SEQ ID NO.: 3, or amino acids 25 – 912 of SEQ ID NO: 18.

In still another particular embodiment, the isolated immunogenic HER-2 peptide has an amino acid sequence that is encoded by a nucleic acid sequence of SEQ ID NO: 4 or 19.

In another aspect, the invention provides an isolated immunogenic HER-2 peptide comprising an ECD-derived peptide, a CTD-derived peptide, and a TMD-derived peptide, wherein the carboxy terminus of the ECD-derived peptide is joined to the amino terminus of the TMD-derived peptide and the carboxy terminus of the TMD-derived peptide is joined to the amino terminus of the CTD-derived peptide. Preferably, the ECD-derived peptide, TMD-derived peptide, and CTD-derived peptide are each joined together by a peptide bond to form a fusion protein,

In some embodiments, the TMD-derived peptide has an amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 16.

In one specific embodiment, the present disclosure provides an isolated immunogenic HER-2 peptide comprising amino acids 23 – 940 of SEQ ID NO: 9; In another specific embodiment, the present disclosure provides an isolated immunogenic HER-2 peptide comprising amino acids 25 – 956 of SEQ ID NO: 20.

The present disclosure also provides functional variants of the immunogenic HER-2 peptides described herein. In some embodiments, the functional variants are at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of any of the specific immunogenic HER-2 peptides provided herein. Functional variants can be obtained by deleting, inserting, or substituting one or more amino acids in a given immunogenic HER-2 peptide. An example for the production of such variants is the conservative substitution of individual amino acids of the polypeptides, that is, by substituting one amino acid by another having similar properties.

In some particular embodiments, the present disclosure provides a functional variant that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of a peptide selected from the group consisting of:

1) a peptide comprising amino acids 23 – 910 of SEQ ID NO: 3;

2) a peptide comprising amino acids 23 – 645 of SEQ ID NO: 5;

3) a peptide comprising amino acids 23 – 940 of SEQ ID NO: 9;

4) a peptide comprising amino acids 25 – 647 of SEQ ID NO: 14;

4) a peptide comprising amino acids 25 – 912 of SEQ ID NO: 18; and

6) a peptide comprising amino acids 25 – 956 of SEQ ID NO: 20.

The isolated immunogenic HER-2 peptides provided by the present invention can be prepared by any suitable method known in the art, such as recombinant technologies.

C. Nucleic Acid Molecules

In other aspects, the present invention provides an isolated nucleic acid molecule that comprises a nucleotide sequence encoding an immunogenic HER-2 peptide, or a functional variant thereof, provided by the present disclosure.

In some embodiments, the nucleotide sequence encodes an ECD-derived peptide provided by the present disclosure. In one embodiment, ECD-derived peptide comprises the amino acid sequences of at least five conserved T cell epitopes of the ECD domain of the human HER-2 protein and shares from 70% to 95% identity, from 75% to 85% identity, or from 85% to 95% identity, with the ECD of the human HER-2 protein.

In another embodiment, the nucleotide sequence encodes an ECD-derived peptide that includes, in positions outside of the epitopes in the ECD of the human HER-2 protein, from 120 to 130 amino acids that are found in one or more of the human HER-2 orthologs and are different from the amino acids found in the ECD of human HER-2 protein.

In still another embodiment, the nucleotide sequence encodes an ECD-derived peptide that includes, in positions outside of the epitopes in the ECD of the human HER-2 protein, from 50 to 130 amino acids that are found in one or more of the human HER-2 orthologs and are different from the amino acids found in the ECD of human HER-2 protein.

In yet another embodiment, the nucleotide sequence encodes an ECD-derived peptide that is joined to a CTD-derived peptide, wherein the CTD-derived peptide comprises the amino acid sequence of the T cell epitope in the CTD domain of the human HER-2 protein and further comprises from 15 to 70 amino acids that are found in one or more of the human HER-2 orthologs and are different from the amino acids found in the CTD of the human HER-2 protein.

In a specific embodiment, the nucleotide sequence encodes an amino acid sequence selected from the group consisting of:

a) amino acids 23 - 910 of SEQ ID NO: 3;

b) amino acids 23 - 645 of SEQ ID NO: 5,

c) amino acids 23 - 940 of SEQ ID NO: 9;

d) amino acids 1- 623 of SEQ ID NO: 12;

e) amino acids 25 - 647 of SEQ ID NO: 14;

f) amino acids 25 - 912 of SEQ ID NO: 18; and

g) amino acids 25 – 956 of SEQ ID NO: 20 .

In another specific embodiment, the nucleotide sequence encodes an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence selected from the group consisting of:

(a) amino acids 23 - 910 of SEQ ID NO: 3;

(b) amino acids 23 - 645 of SEQ ID NO: 5,

(c) amino acids 23 - 940 of SEQ ID NO: 9;

(d) amino acids 1- 623 of SEQ ID NO: 12;

(e) amino acids 25 - 647 of SEQ ID NO: 14;

(f) amino acids 25 - 912 of SEQ ID NO: 18; and

(g) amino acids 25 – 956 of SEQ ID NO: 20.

In some further embodiments, the isolated nucleic acid molecule described herein above further comprises a nucleotide sequence that encodes the amino acids of the signal sequence of human HER-2 protein. The amino acid sequence of the signal sequence corresponds to amino acids 1 - 22 of SEQ ID NOs: 1, 3, 5, or 9. Codons encoding additional amino acids may be inserted into the nucleotide sequence in order to facilitate the expression of the protein. For example, codons encoding alanine and serine may be inserted into the nucleotide sequence at the positions between amino acid positions 1 and 2 of the signal sequence. One example of such a modified signal sequence is an amino acid sequence that corresponds to amino acids 1-24 of SEQ ID NO: 14, 18, and 20.

In another specific embodiment, the present invention provides a nucleic acid molecule that comprises the nucleotide sequence of SEQ IDS NO.: 4, 6, 10, 13, 15, 19, or 21. According to another aspect of the invention, there is provided a plasmid construct capable of expressing an immunogenic HER-2 peptide, which comprises a nucleic acid molecule provided by the invention inserted into an expression vector.

Useful vectors include, but not limited to, biodegradable microcapsules, immuno-stimulating complexes (ISCOMs) or liposomes, and genetically engineered attenuated live vectors such as viruses or bacteria. Examples of suitable attenuated live bacterial vectors include Salmonella typhimurium, Salmonella typhi, Shigella, Bacillus, Lactobacillus, Bacille Calmette-Guerin (BCG), Escherichia coli, Vibrio cholerae, Campylobacter, Listeria monocytogenes, or any other suitable bacterial vector, as is known in the art. Methods of transforming live bacterial vectors with an exogenous DNA construct are well described in the art. See, for example, Joseph Sambrook and David W. Russell, Molecular Cloning, A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001). Examples of suitable viral vectors include bacteriophages, herpes virus, adenovirus, polio virus, vaccinia virus, and avipox. Methods of transforming viral vector with an exogenous DNA construct are also well described in the art.. Individual expression vectors capable of expressing the genetic material can be produced using standard recombinant techniques. General cloning methods are described in, e.g., Maniatis et al., 1985 Molecular Cloning: A Laboratory Manual or DNA Cloning, Vol. I and II (D. N. Glover, ed., 1985).

D. Compositions Comprising an Immunogenic HER-2 Peptide (Peptide Compositions)

In another aspect of the present disclosure, there is provided a composition which comprises an isolated immunogenic HER-2 peptide provided by the present disclosure. The peptide compositions are useful for eliciting an immune response against HER-2 protein in a mammal, such as a human. In some embodiments, the composition is a peptide vaccine composition useful for immunization of a mammal for inhibiting abnormal cell proliferation, for providing protection against the development of cancer (used as a prophylactic), or for treatment of disorders (used as a therapeutic) associated with the HER-2 over expression, such as cancer.

In one embodiment, the peptide composition comprises an isolated immunogenic HER-2 peptide selected from the group consisting of:

(a) an ECD-derived peptide that comprises the amino acid sequence of at least four, at least five, or at least six conserved T cell epitopes of the ECD domain of the human HER-2 and shares from 70% to 95% identity, from 75% to 85% identity, or from 85% to 95% identity, with the ECD of human HER-2 protein;

(b) an ECD-derived peptide that includes, in positions outside of the conserved T cell epitopes in the ECD of the human HER-2, from 50 to 130 amino acids that are

found in one or more of the human HER-2 orthologs and are different from the amino acids found in the ECD of human HER-2;

(c) an ECD-derived peptide that includes, in positions outside of the conserved T cell epitopes in the ECD of the human HER-2, from 120 to 130 amino acids that are found in one or more of the human HER-2 orthologs and are different from the amino acids found in the ECD of human HER-2;

(d) an ECD-derived peptide that includes, in positions outside of the conserved T cell epitopes in the ECD of the human HER-2, from 50 to 70 amino acids that are found in one or more of the human HER-2 orthologs and are different from the amino acids found in the ECD of human HER-2;

(e) a peptide comprisign an ECD-derived peptide provided by the disclosure and a CTD-derived peptide provided by the disclosure, wherein the C-terminal end of the ECD-derived peptide is joined by a peptide bond to the N-terminal end of the CTD-derived peptide;

(f) a peptide comprising amino acids 23 – 645 of SEQ ID NO: 5;

(g) a peptide comprising amino acids 23 – 910 of SEQ ID NO: 3;

(h) a peptide comprising an ECD-derived peptide provided by the disclosure, a CTD-derived peptide provided by the disclosure, and an amino acid sequence of SEQ ID NO: 11, wherein the carboxy terminus of the ECD-derived peptide is joined to the amino terminus of the amino acid sequence of SEQ ID NO: 11 and the carboxy terminus of the amino acid sequence of SEQ ID NO: 11 is joined to the amino terminus of the CTD-derived peptide;

(i) a peptide comprising amino acids 23 – 940 of SEQ ID NO: 9.

(j) a peptide comprising an amino acids 25 – 647 of SEQ ID NO: 14;

(k) a peptide comprising an amino acids 25 – 912 of SEQ ID NO: 18; and

(l) a peptide comprising an amino acids 25 – 956 of SEQ ID NO: 20.

In another aspect, the peptide composition further comprises one or more other tumor-associated antigens (TAAs). Examples of other TAA include Survivin, WT1, MUC1, CEA, NY-ESO-1, MAGE, MART-1 and other antigens disclosed in the article "The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research" (Cheever MA, Clin Cancer Res.15(17), 5323, 2009).

The peptide composition may further comprise a pharmaceutically acceptable excipient. Examples of suitable excipients include biocompatible oils, such as rape seed

oil, sunflower oil, peanut oil, cotton seed oil, jojoba oil, squalan or squalene, physiological saline solution, preservatives and osmotic pressure controlling agents, carrier gases, pH-controlling agents, organic solvents, hydrophobic agents, enzyme inhibitors, water absorbing polymers, surfactants, absorption promoters, pH modifiers,, and anti-oxidative agents.

In some embodiments, the peptide composition is a vaccine composition. The immunogenic HER-2 peptide in a composition, particularly a vaccine composition, of the invention may be linked to, conjugated to, or otherwise incorporated into a carrier for administration to a patient for systemic immunization. The term "carrier" refers to a substance or structure that an immunogen can be attached to or otherwise associated with for delivery of the immunogen to the recipient (e.g., patient). The carrier itself may be immunogenic. Examples of carriers include immunogenic peptides, immune CpG islands, limpet hemocyanin (KLH), tetanus toxoid (TT), cholera toxin subunit B (CTB), bacteria or bacterial ghosts, liposome, chitosome, virosomes, microspheres, dendritic cells, or their like. The immunogenic peptide can be conjugated to the carrier in a single or multiple ways in different combinations as mono-, di-, tri or oligomers. Such conjugations are known in the art, see for example: Th. H. Turpen, S. J. Reinl, Y. Charoenvit, S. L. Hoffmann, V. Fallarme in Bio/Technology, 1995, Vol. 13, pages 53 to 57, by examples of the conjugation of epitopes to macromolecular carriers, or by Wagner et al, 2005 J. Immunol. 174:976-982.

The vaccine composition may be used in conjunction with one or more adjuvants. The adjuvants may be formulated separately from the vaccine composition, or they may be part of the same vaccine composition formulation. Thus, in one embodiment, the vaccine composition further comprises one or more adjuvants. Suitable adjuvants include those suitable for use in mammals, preferably in humans. Examples of known suitable adjuvants include, but are not limited to, aluminum salts (such as alum, aluminum phosphate, aluminum hydroxide), CpG-containing nucleic acids (where the cytosine is unmethylated), QS21 (saponin adjuvant), MPL (Monophosphoryl Lipid A), 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS (see, e.g., Sjölander et al., *J. Leukocyte Biol.* 64:713 (1998); PCT Publication Nos. WO 90/03184, WO 96/11711, WO 00/48630, WO 98/36772, WO 00/41720, WO 06/134423 and WO 07/026190), LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, Quil A, interleukins, Freund's, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-

acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. Further exemplary adjuvants include, but are not limited to: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (PCT Publication No. WO 90/14837; Chapter 10 in *Vaccine design: the subunit and adjuvant approach,* eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80 (polyoxyethylene sorbitan mono-oleate), and 0.5% Span 85 (sorbitan trioleate) (optionally containing muramyl tri-peptide covalently linked to dipalmitoyl phosphatidylethanolamine (MTP-PE)) formulated into submicron particles using a microfluidizer, (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RIBI™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components such as monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DETOX™); (2) saponin adjuvants, such as QS21, STIMULON™ (Cambridge Bioscience, Worcester, MA), Abisco® (Isconova, Sweden), or Iscomatrix® (Commonwealth Serum Laboratories, Australia), may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS may be devoid of additional detergent *e.g.* PCT Publication No. WO 00/07621; (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins *(e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (PCT Publication No. WO 99/44636), *etc.),* interferons *(e.g.* gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), *etc.;* (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL), optionally in the substantial absence of alum when used with pneumococcal saccharides (*e.g.* GB-2220221, EP-A-0689454, WO 00/56358); (6) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (*e.g.* EP-A-0835318, EP-A-0735898, EP-A-0761231); (7) oligonucleotides comprising CpG motifs , *i.e.* containing at least one CG dinucleotide, where the cytosine is unmethylated (e.g., Krieg, *Vaccine* (2000) 19:618-622; Krieg, *Curr Opin Mol Ther* (2001) 3:15-24; WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581); (8) a polyoxyethylene ether

or a polyoxyethylene ester (*e.g.* WO 99/52549); (9) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (e.g., WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (e.g., WO 01/21152); (10) a saponin and an immunostimulatory oligonucleotide *(e.g.* a CpG oligonucleotide) (e.g., WO 00/62800); (11) an immunostimulant and a particle of metal salt (*e.g.* WO 00/23105); (12) a saponin and an oil-in-water emulsion (*e.g.* WO 99/11241); (13) a saponin *(e.g.* QS21) + 3dMPL + IM2 (optionally + a sterol)(*e.g.* WO 98/57659); (14) other substances that act as immunostimulating agents to enhance the efficacy of the composition, such as Muramyl peptides including N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-25 acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutarninyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), (15) ligands for toll-like receptors (TLR), natural or synthesized (e.g. Kanzler et al., *Nature Med*. 13:1552-1559 (2007)), including TLR3 ligands such as polyl:C and similar compounds such as Hiltonol and Ampligen.

The peptide vaccine compositions can be prepared by methods known to one skilled in the art. Typically, such compositions may be prepared as injectables, either as liquid solutions, suspensions, or emulsions. The immunogenic peptide can also be encapsulated in liposomes. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5 to 10 percent, preferably 1 to 2 percent. Oral formulations may include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions may take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10 percent to 95 percent of effective ingredient, preferably 25 to 70 percent.

The peptide vaccine compositions provide by the present invention may be administered by any suitable route, such as by injection, either subcutaneously or intramuscularly. It can also be administered in other manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective.

The peptide vaccine compositions can be administered in a single dose schedule, or in a multiple dose schedule. A multiple dose schedule is one in which a primary

course of vaccination can include 1 or more separate doses, followed by other doses given at subsequent time intervals required to maintain and or reinforce the immune response, for example, at 1 to 4 months for a second dose, and if needed, a subsequent dose(s) after several months. Periodic boosters at intervals of 1 to 5 years, usually 3 years, are desirable to maintain the desired levels of protective immunity.

E. Compositions Comprising a Nucleic Acid Molecule (DNA Compositions)

The present invention also provides a composition comprising an isolated nucleic acid molecule provided by the present disclosure. The DNA compositions are useful for eliciting an immune response against HER-2 protein in a mammal including a human. In some embodiments, the DNA compositions are DNA vaccine compositions useful for inhibiting abnormal cell proliferation, providing protection against the development of cancer (used as a prophylactic), or for treatment of cancer (used as a therapeutic) associated with the HER-2 over expression.

In some specific embodiments, the nucleic acid molecule in a DNA composition comprises a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of:

a) amino acids 23 - 910 of SEQ ID NO: 3;

b) amino acids 23 - 645 of SEQ ID NO: 5,

c) amino acids 23 - 940 of SEQ ID NO: 9;

d) amino acids 1- 623 of SEQ ID NO: 12;

e) amino acids 25 - 647 of SEQ ID NO: 14;

f) amino acids 25 - 912 of SEQ ID NO: 18; and

g) amino acids 25 – 956 of SEQ ID NO.: 20 .

In some further embodiments, the isolated nucleic acid molecule in the DNA composition described herein above further comprises a nucleotide sequence that encodes the amino acids of the signal sequence of human HER-2 protein. The amino acid sequence of the signal sequence corresponds to amino acids 1 - 22 of SEQ ID NOs: 1, 3, 5, or 9. Codons encoding additional amino acids may be inserted into the nucleotide sequence in order to facilitate the expression of the protein. For example, codons encoding alanine and serine may be inserted into the nucleotide sequence at the positions between amino acid positions 1 and 2 of the signal sequence. One example of such a modified signal sequence is an amino acid sequence that corresponds to amino acids 1-24 of SEQ ID NO: 14, 18, and 20.

In another specific embodiment, the nucleic acid molecule in the DNA composition comprises the nucleotide sequence of SEQ IDS NO: 4, 6, 10, 13, 15, 19, or 21.

In other embodiments, the DNA compositions are DNA vaccine compositions. The DNA compositions, including the DNA vaccine compositions, may further comprise a pharmaceutically acceptable excipient. Suitable pharmaceutically acceptable excipients for DNA compositions, including DNA vaccine compositions, are well known to those skilled in the art and include but are not limited to proteins, sugars, etc. Such excipients may be aqueous or non aqueous solutions, suspensions, and emulsions. Examples of non-aqueous excipients include propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Examples of aqueous excipient include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Suitable excipients also include agents that assist in cellular uptake of the polynucleotide molecule. Examples of such agents are (i) chemicals that modify cellular permeability, such as bupivacaine, (ii) liposomes or viral particles for encapsulation of the polynucleotide, or (iii) cationic lipids or silica, gold, or tungsten microparticles which associate themselves with the polynucleotides. Anionic and neutral liposomes are well-known in the art (see, e.g., Liposomes: A Practical Approach, RPC New Ed, IRL press (1990), for a detailed description of methods for making liposomes) and are useful for delivering a large range of products, including polynucleotides. Cationic lipids are also known in the art and are commonly used for gene delivery. Such lipids include Lipofectin.TM. also known as DOTMA (N-[l-(2,3-dioleyloxy) propyls N,N, N-trimethylammonium chloride), DOTAP (1,2-bis (oleyloxy)-3 (trimethylammonio) propane), DDAB (dimethyldioctadecyl-ammonium bromide), DOGS (dioctadecylamidologlycyl spermine) and cholesterol derivatives such as DCChoi (3 beta-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol). A description of these cationic lipids can be found in EP 187,702, WO 90/11092, U.S. Pat. No. 5,283,185, WO 91/15501, WO 95/26356, and U.S. Pat. No. 5,527,928. Cationic lipids for gene delivery are preferably used in association with a neutral lipid such as DOPE (dioleyl phosphatidylethanolamine), as described in WO 90/11092 as an example.

The nucleic acid molecule in the DNA vaccine composition may be a ‘naked” nucleic acid molecule, i.e. simply in the form of an isolated DNA. Alternatively, the nucleic acid molecule can be incorporated into a vector. Examples of suitable vectors

include those described herein above. The DNA compositions, including the vaccine compositions, can be introduced into tissues of an animal, including human, by a number of methods known in the art. Examples of suitable methods include: (1) injection with hypodermic needle, such as intramuscular (IM), intradermal (ID), intravenous, subcutaneous, and intraperitoneal injection; (2) pneumatic (jet) injection, gene gun injection, topical application (such as ocular and intravaginal application) , and liposome-mediated delivery.

One particular method that may be used is gene gun delivery using the Particle Mediated Epidermal Delivery (PMED™) vaccine delivery device marketed by PowderMed. PMED is a needle-free method of administering vaccines to animals or to patients. The PMED system involves the precipitation of DNA onto microscopic gold particles that are then propelled by helium gas into the epidermis. The DNA-coated gold particles are delivered into the antigen-presenting cells (APCs) of the epidermis, and once inside the nuclei of the APCs, the DNA elutes off the gold and becomes transcriptionally active, producing encoded protein. This protein is then presented by the APCs to the lymphocytes to induce a T-cell-mediated immune response. The use of gold or tungsten microparticles used for gene delivery is also known in the art (see for example, WO 91/00359 and WO 93/17706). The microparticle-coated polynucleotide is injected via intradermal or intraepidermal routes using a needleless injection device, such as those described in U.S. Pat. No. 4,945,050, U.S. Pat. No. 5,015,580, and WO 94/24263.

F. Uses of the Compositions

In another aspect, the present disclosure provides a method of eliciting an immune response against HER-2 protein in a mammal, particularly a human, comprising administering to the mammal an effective amount of a peptide composition, or an effective amount of a DNA composition, provided by the present disclosure.

In another aspect, the present disclosure provides a method of treating cancer in a human subject associated with overexpression of HER-2 protein. The method comprises administering to the human subject an effective amount of a peptide composition, or a DNA composition, provided by the present disclosure. Examples of cancers that may be treated with the method include breast cancer, stomach cancer, ovarian cancer, lung cancer, bladder cancer, and prostate cancer.

In another aspect, the present disclosure provides a method of preventing cancer in a human associated with over expression of HER-2 protein. The method comprises

administering to a human subject an effective amount of a peptide composition, or a DNA composition, provided by the present disclosure, wherein the human subject is at increased risk of developing a cancer associated with over expression of HER-2 protein. Examples of cancers that may be prevented with the method include breast cancer, stomach cancer, ovarian cancer, lung cancer, bladder cancer, and prostate cancer.

In still another aspect, the present disclosure provides a method of inhibiting abnormal cell proliferation in a human subject, comprising administering to the human subject an effective amount of a peptide composition, or a DNA composition, provided by the present disclosure.

The effective amount of the peptide or DNA in the composition, such as a vaccine composition, to be administered can be readily determined by a person skilled in the art, and will depend a number of factors, such as: (1) the subject to be treated, including the subject's the immune status and health, (2) the specific condition to be treated, (3) the specific active therapeutic agent used, (4) the degree of protection or treatment desired, (5) the administration schedule, and (6) whether any other therapeutic agents are used, and the therapeutic activity of the particular polypeptide. Suitable dosage ranges are of the order of several hundred micrograms effective ingredient per vaccination with a range from about 0.01 to 10 mg/kg/day, preferably in the range from about 0.1 to 1 mg/kg/day.

Table 5. Raw Sequence Listing
(Signal sequence underlined)

SEQ ID NO: 1: Amino Acid Sequence of Full Length Human HER-2 Protein

MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFLQDIQ
EVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQ
RNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGESSEDCQSLTRTVCAGGCARCKGPLPTD
CCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSCT
LVCPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTA
PLQPEQLQVFETLEEITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLALI
HHNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDECVGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQECVEECRV
LQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQ
PCPINCTHSCVDLDDKGCPAEQRASPLTSIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRRLLQETELVEPLTP
SGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENTSPKANKEILDEAYVMAGVGSP
YVSRLLGICLTSTVQLVTQLMPYGCLLDHVRENRGRLGSQDLLNWCMQIAKGMSYLEDVRLVHRDLAARNVLVKSPNH

VKITDFGLARLLDIDETEYHADGGKVPIKWMALESILRRRFTHQSDVWSYGVTVWELMTFGAKPYDGIPAREIPDLLE
KGERLPQPPICTIDVYMIMVKCWMIDSECRPRFRELVSEFSRMARDPQRFVVIQNEDLGPASPLDSTFYRSLLEDDDM
GDLVDAEEYLVPQQGFFCPDPAPGAGGMVHHRHRSSSTRSGGGDLTLGLEPSEEEAPRSPLAPSEGAGSDVFDGDLGM
GAAKGLQSLPTHDPSPLQRYSEDPTVPLPSETDGYVAPLTCSPQPEYVNQPDVRPQPPSPREGPLPAARPAGATLERP
KTLSPGKNGVVKDVFAFGGAVENPEYLTPQGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEY
LGLDVPV

SEQ ID NO: 2: DNA Sequence Encoding Full Length Human HER-2 Protein of SEQ ID NO: 1

ATGGAGCTGGCGGCCTTGTGCCGCTGGGGGCTCCTCCTCGCCCTCTTGCCCCCCGGAGCCGCGAGCACCCAAGTGTGC
ACCGGCACAGACATGAAGCTGCGGCTCCCTGCCAGTCCCGAGACCCACCTGGACATGCTCCGCCACCTCTACCAGGGC
TGCCAGGTGGTGCAGGGAAACCTGGAACTCACCTACCTGCCCACCAATGCCAGCCTGTCCTTCCTGCAGGATATCCAG
GAGGTGCAGGGCTACGTGCTCATCGCTCACAACCAAGTGAGGCAGGTCCCACTGCAGAGGCTGCGGATTGTGCGAGGC
ACCCAGCTCTTTGAGGACAACTATGCCCTGGCCGTGCTAGACAATGGAGACCCGCTGAACAATACCACCCCTGTCACA
GGGGCCTCCCCAGGAGGCCTGCGGGAGCTGCAGCTTCGAAGCCTCACAGAGATCTTGAAAGGAGGGGTCTTGATCCAG
CGGAACCCCCAGCTCTGCTACCAGGACACGATTTTGTGGAAGGACATCTTCCACAAGAACAACCAGCTGGCTCTCACA
CTGATAGACACCAACCGCTCTCGGGCCTGCCACCCCTGTTCTCCGATGTGTAAGGGCTCCCGCTGCTGGGGAGAGAGT
TCTGAGGATTGTCAGAGCCTGACGCGCACTGTCTGTGCCGGTGGCTGTGCCCGCTGCAAGGGGCCACTGCCCACTGAC
TGCTGCCATGAGCAGTGTGCTGCCGGCTGCACGGGCCCCAAGCACTCTGACTGCCTGGCCTGCCTCCACTTCAACCAC
AGTGGCATCTGTGAGCTGCACTGCCCAGCCCTGGTCACCTACAACACAGACACGTTTGAGTCCATGCCCAATCCCGAG
GGCCGGTATACATTCGGCGCCAGCTGTGTGACTGCCTGTCCCTACAACTACCTTTCTACGGACGTGGGATCCTGCACC
CTCGTCTGCCCCCTGCACAACCAAGAGGTGACAGCAGAGGATGGAACACAGCGGTGTGAGAAGTGCAGCAAGCCCTGT
GCCCGAGTGTGCTATGGTCTGGGCATGGAGCACTTGCGAGAGGTGAGGGCAGTTACCAGTGCCAATATCCAGGAGTTT
GCTGGCTGCAAGAAGATCTTTGGGAGCCTGGCATTTCTGCCGGAGAGCTTTGATGGGGACCCAGCCTCCAACACTGCC
CCGCTCCAGCCAGAGCAGCTCCAAGTGTTTGAGACTCTGGAAGAGATCACAGGTTACCTATACATCTCAGCATGGCCG
GACAGCCTGCCTGACCTCAGCGTCTTCCAGAACCTGCAAGTAATCCGGGGACGAATTCTGCACAATGGCGCCTACTCG
CTGACCCTGCAAGGGCTGGGCATCAGCTGGCTGGGGCTGCGCTCACTGAGGGAACTGGGCAGTGGACTGGCCCTCATC
CACCATAACACCCACCTCTGCTTCGTGCACACGGTGCCCTGGGACCAGCTCTTTCGGAACCCGCACCAAGCTCTGCTC
CACACTGCCAACCGGCCAGAGGACGAGTGTGTGGGCGAGGGCCTGGCCTGCCACCAGCTGTGCGCCCGAGGGCACTGC
TGGGGTCCAGGGCCCACCCAGTGTGTCAACTGCAGCCAGTTCCTTCGGGGCCAGGAGTGCGTGGAGGAATGCCGAGTA
CTGCAGGGGCTCCCCAGGGAGTATGTGAATGCCAGGCACTGTTTGCCGTGCCACCCTGAGTGTCAGCCCCAGAATGGC
TCAGTGACCTGTTTTGGACCGGAGGCTGACCAGTGTGTGGCCTGTGCCCACTATAAGGACCCTCCCTTCTGCGTGGCC
CGCTGCCCCAGCGGTGTGAAACCTGACCTCTCCTACATGCCCATCTGGAAGTTTCCAGATGAGGAGGGCGCATGCCAG
CCTTGCCCCATCAACTGCACCCACTCCTGTGTGGACCTGGATGACAAGGGCTGCCCCGCCGAGCAGAGAGCCAGCCCT
CTGACGTCCATCATCTCTGCGGTGGTTGGCATTCTGCTGGTCGTGGTCTTGGGGGTGGTCTTTGGGATCCTCATCAAG
CGACGGCAGCAGAAGATCCGGAAGTACACGATGCGGAGACTGCTGCAGGAAACGGAGCTGGTGGAGCCGCTGACACCT
AGCGGAGCGATGCCCAACCAGGCGCAGATGCGGATCCTGAAAGAGACGGAGCTGAGGAAGGTGAAGGTGCTTGGATCT
GGCGCTTTTGGCACAGTCTACAAGGGCATCTGGATCCCTGATGGGGAGAATGTGAAAATTCCAGTGGCCATCAAAGTG
TTGAGGGAAAACACATCCCCCAAAGCCAACAAAGAAATCTTAGACGAAGCATACGTGATGGCTGGTGTGGGCTCCCCA
TATGTCTCCCGCCTTCTGGGCATCTGCCTGACATCCACGGTGCAGCTGGTGACACAGCTTATGCCCTATGGCTGCCTC
TTAGACCATGTCCGGGAAAACCGCGGACGCCTGGGCTCCCAGGACCTGCTGAACTGGTGTATGCAGATTGCCAAGGGG

ATGAGCTACCTGGAGGATGTGCGGCTCGTACACAGGGACTTGGCCGCTCGGAACGTGCTGGTCAAGAGTCCCAACCAT
GTCAAAATTACAGACTTCGGGCTGGCTCGGCTGCTGGACATTGACGAGACAGAGTACCATGCAGATGGGGGCAAGGTG
CCCATCAAGTGGATGGCGCTGGAGTCCATTCTCCGCCGGCGGTTCACCCACCAGAGTGATGTGTGGAGTTATGGTGTG
ACTGTGTGGGAGCTGATGACTTTTGGGGCCAAACCTTACGATGGGATCCCAGCCCGGGAGATCCCTGACCTGCTGGAA
AAGGGGGAGCGGCTGCCCCAGCCCCCCATCTGCACCATTGATGTCTACATGATCATGGTCAAATGTTGGATGATTGAC
TCTGAATGTCGGCCAAGATTCCGGGAGTTGGTGTCTGAATTCTCCCGCATGGCCAGGGACCCCCAGCGCTTTGTGGTC
ATCCAGAATGAGGACTTGGGCCCAGCCAGTCCCTTGGACAGCACCTTCTACCGCTCACTGCTGGAGGACGATGACATG
GGGGACCTGGTGGATGCTGAGGAGTATCTGGTACCCCAGCAGGGCTTCTTCTGTCCAGACCCTGCCCCGGGCGCTGGG
GGCATGGTCCACCACAGGCACCGCAGCTCATCTACCAGGAGTGGCGGTGGGGACCTGACACTAGGGCTGGAGCCCTCT
GAAGAGGAGGCCCCCAGGTCTCCACTGGCACCCTCCGAAGGGGCTGGCTCCGATGTATTTGATGGTGACCTGGGAATG
GGGGCAGCCAAGGGGCTGCAAAGCCTCCCCACACATGACCCCAGCCCTCTACAGCGGTACAGTGAGGACCCCACAGTA
CCCCTGCCCTCTGAGACTGATGGCTACGTTGCCCCCCTGACCTGCAGCCCCCAGCCTGAATATGTGAACCAGCCAGAT
GTTCGGCCCCAGCCCCCTTCGCCCCGAGAGGGCCCTCTGCCTGCTGCCCGACCTGCTGGTGCCACTCTGGAAAGGCCC
AAGACTCTCTCCCCAGGGAAGAATGGGGTCGTCAAAGACGTTTTTGCCTTTGGGGGTGCCGTGGAGAACCCCGAGTAC
TTGACACCCCAGGGAGGAGCTGCCCCTCAGCCCCACCCTCCTCCTGCCTTCAGCCCAGCCTTCGACAACCTCTATTAC
TGGGACCAGGACCCACCAGAGCGGGGGGCTCCACCCAGCACCTTCAAAGGGACACCTACGGCAGAGAACCCAGAGTAC
CTGGGTCTGGACGTGCCAGTG

SEQ ID No: 3: Amino Acid Sequence of RaDHER2-1 Peptide

MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDIVRHLYQGCQVVQGNLELTYVPANASLSFLQDIQ
EVQGYMLIAHSRVKHIPLQRLRIVRGTQLFEDNYALAVLDNRDLQDNATSAAGRTPEGLRELQLRSLTEILKGGVLIR
GSPQLCHQDMVLWEDVLRKNNQLTPVDMDTNRSRACPPCAPACRDNHCWGASPGDCNSLTGTICTSGCARCKGRQPTD
CCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPSLIIYNTDTFESMHNPEGRYTFGASCVTTCPYNYLSTEVGSCT
LVCPPNNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLRGARAITSDNVQDFVGCKKIFGSLAFLPESFDGDPSSGIA
PLRPEHLRVFEALEEITGYLYISAWPESFRNLSVLQNLRIIRGRVLHDGAYSLALQGLGIRSLGLRSLQELGSGLALV
HRNARLCFVNTVPWAQLFRNPHQALLHSGNPSEDECGLKDFVCNSLCAHGHCWGPGPTHCVNCSQFLPGQECVKECRV
WKGLPREYVSDKRCLPCHSECQPQNSTETCYGSEADQCEACTHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQ
PCPINCTHSCADLDDRGCPAENEDLGPSSPMDSTFYRSLLEDEDMGELVDAEEYLVPQQGFFSPDPTPGTGSTAHRRH
RSSSARNGGGDLTLGMEPSGEGPPRSPRAPSEGTGSDVFDGDLAVGVTKGLQSLSPQDLSPLQRYSEDPTLPLPSETD
GKVAPLSCSPQPEFVNQSDVQPKSPLTPEGPPSPARPTGATLERAKTLSPGKNGVVKDVFTFGGAVENPEFLAPREGT
ASPPHPSPAFSPAFDNLFFWDQNSSEQGPPPSNFEGTPTAENPEFLGLDVPV.

SEQ ID NO: 4: DNA Sequence Encoding the RaDHER2-1 Peptide of SEQ ID No: 3

ATGGAACTGGCCGCCCTGTGTAGATGGGGACTGCTGCTGGCCCTGCTGCCCCCTGGCGCTGCTTCCACACAGGTGTGC
ACCGGCACCGACATGAAGCTGAGACTGCCCGCCAGCCCTGAGACCCACCTGGACATCGTGCGGCACCTGTACCAGGGC
TGTCAGGTGGTGCAGGGCAACCTGGAACTGACCTACGTGCCCGCCAACGCCAGCCTGAGCTTCCTGCAGGACATCCAG
GAAGTGCAGGGCTACATGCTGATCGCCCACAGCCGGGTGAAGCACATCCCCCTGCAGCGGCTGAGAATCGTGCGGGGC
ACCCAGCTGTTCGAGGACAACTACGCCCTGGCCGTGCTGGACAACCGGGACCTGCAGGATAATGCCACCTCCGCCGCT
GGCAGAACACCTGAGGGCCTGCGGGAGCTGCAGCTGAGAAGCCTGACCGAGATCCTGAAGGGCGGCGTGCTGATCAGA
GGCAGCCCCCAGCTGTGCCATCAGGATATGGTGCTGTGGGAGGACGTGCTGCGGAAGAACAACCAGCTGACCCCCGTG

GACATGGACACCAACCGGTCCAGAGCCTGCCCTCCTTGCGCCCCTGCCTGCAGGGATAACCACTGCTGGGGCGCCAGC
CCAGGCGATTGCAACAGCCTGACCGGCACCATCTGCACCAGCGGCTGCGCCAGATGCAAGGGCAGACAGCCCACCGAC
TGCTGCCATGAGCAGTGTGCCGCCGGATGTACCGGCCCCAAGCACAGCGACTGCCTGGCCTGCCTGCACTTCAACCAC
AGCGGCATCTGCGAGCTGCACTGCCCCAGCCTGATCATCTACAACACCGACACCTTCGAGAGCATGCACAACCCCGAG
GGCAGATACACCTTCGGCGCCAGCTGCGTGACCACCTGCCCCTACAACTACCTGAGCACCGAAGTGGGCAGCTGCACC
CTGGTGTGCCCCCCCAACAACCAGGAAGTGACCGCCGAGGACGGCACCCAGAGATGCGAGAAGTGCAGCAAGCCCTGC
GCCAGAGTGTGTTACGGCCTGGGCATGGAACATCTGAGGGGCGCCAGGGCCATCACCAGCGACAACGTGCAGGACTTC
GTGGGCTGCAAGAAGATTTTCGGCTCCCTGGCCTTCCTGCCCGAGAGCTTCGACGGCGACCCTAGCAGCGGCATCGCC
CCCCTGAGACCAGAGCACCTGCGGGTGTTCGAGGCCCTGGAAGAGATCACCGGCTACCTGTACATCAGCGCCTGGCCC
GAGTCCTTCCGGAACCTGAGCGTGCTGCAGAACCTGCGGATCATCCGGGGCAGAGTGCTGCACGATGGCGCCTATAGC
CTCGCTCTGCAGGGACTGGGAATCAGAAGCCTGGGCCTGCGGTCTCTGCAGGAACTGGGCAGCGGACTGGCCCTGGTG
CACCGGAACGCCCGGCTGTGCTTCGTGAATACCGTGCCCTGGGCCCAGCTGTTTAGGAACCCCCACCAGGCTCTGCTG
CACAGCGGCAACCCCAGCGAGGACGAGTGCGGCCTGAAGGACTTTGTGTGCAACTCCCTGTGCGCCCACGGACACTGT
TGGGGACCTGGACCTACCCACTGCGTGAACTGCAGCCAGTTTCTGCCTGGCCAGGAATGCGTGAAAGAATGCAGAGTG
TGGAAGGGCCTGCCTCGGGAGTACGTGAGCGACAAGCGGTGCCTGCCCTGCCACAGCGAGTGCCAGCCCCAGAACAGC
ACCGAGACCTGCTACGGCAGCGAGGCCGACCAGTGTGAGGCCTGCACCCACTACAAGGACCCCCCCTTCTGCGTGGCC
AGATGCCCTAGCGGCGTGAAGCCCGACCTGAGCTACATGCCCATCTGGAAGTTCCCCGACGAGGAAGGCGCCTGCCAG
CCCTGCCCCATCAACTGCACCCACAGCTGCGCCGACCTGGACGATAGAGGCTGCCCTGCCGAGAACGAGGATCTGGGC
CCCAGCAGCCCTATGGACAGCACCTTCTACAGATCCCTGCTGGAAGATGAGGACATGGGCGAACTGGTGGACGCCGAG
GAATACCTGGTGCCTCAGCAGGGCTTCTTCAGCCCCGATCCTACCCCTGGCACCGGCAGCACAGCCCATCGGCGGCAC
AGAAGCAGTTCTGCTAGAAATGGCGGCGGAGACCTGACCCTGGGAATGGAACCTAGCGGCGAGGGCCCTCCTAGAAGC
CCTAGAGCACCTTCCGAAGGGACCGGCTCCGACGTGTTCGATGGCGATCTGGCCGTGGGCGTGACAAAGGGCCTGCAG
TCTCTCTCTCCACAGGATCTGTCTCCACTGCAGAGATACAGCGAGGACCCCACCCTGCCTCTGCCTAGCGAGACCGAC
GGCAAGGTGGCCCCTCTGAGCTGTAGCCCCCAGCCCGAGTTCGTGAACCAGAGCGACGTGCAGCCCAAGAGCCCTCTG
ACCCCTGAGGGACCCCCTAGCCCTGCCAGACCTACCGGCGCCACCCTGGAAAGAGCCAAGACCCTGAGCCCCGGCAAG
AACGGCGTGGTGAAGGACGTGTTCACCTTTGGCGGAGCCGTGGAGAACCCTGAGTTCCTGGCCCCAAGAGAGGGCACA
GCCAGCCCTCCTCACCCCAGCCCAGCCTTCAGCCCTGCCTTCGACAACCTGTTCTTCTGGGACCAGAATTCTAGTGAA
CAGGGACCTCCACCCAGCAATTTCGAGGGCACCCCCACCGCCGAGAATCCCGAGTTTCTGGGCCTGGACGTGCCCGTG
TAG

SEQ ID NO:5: Amino Acid Sequence of ECD-derived Peptide 1(ECD1)

MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDIVRHLYQGCQVVQGNLELTYVPANASLSFLQDIQ
EVQGYMLIAHSRVKHIPLQRLRIVRGTQLFEDNYALAVLDNRDLQDNATSAAGRTPEGLRELQLRSLTEILKGGVLIR
GSPQLCHQDMVLWEDVLRKNNQLTPVDMDTNRSRACPPCAPACRDNHCWGASPGDCNSLTGTICTSGCARCKGRQPTD
CCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPSLIIYNTDTFESMHNPEGRYTFGASCVTTCPYNYLSTEVGSCT
LVCPPNNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLRGARAITSDNVQDFVGCKKIFGSLAFLPESFDGDPSSGIA
PLRPEHLRVFEALEEITGYLYISAWPESFRNLSVLQNLRIIRGRVLHDGAYSLALQGLGIRSLGLRSLQELGSGLALV
HRNARLCFVNTVPWAQLFRNPHQALLHSGNPSEDECGLKDFVCNSLCAHGHCWGPGPTHCVNCSQFLPGQECVKECRV
WKGLPREYVSDKRCLPCHSECQPQNSTETCYGSEADQCEACTHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQ
PCPINCTHSCADLDDRGCPAE

SEQ ID NO:6: DNA Sequence Encoding the Amino Acid sequence of ECD1 (SEQ ID NO:5)

ATGGAACTGGCCGCCCTGTGTAGATGGGGACTGCTGCTGGCCCTGCTGCCCCCTGGCGCTGCTTCCACACAGGTGTGC
ACCGGCACCGACATGAAGCTGAGACTGCCCGCCAGCCCTGAGACCCACCTGGACATCGTGCGGCACCTGTACCAGGGC
TGTCAGGTGGTGCAGGGCAACCTGGAACTGACCTACGTGCCCGCCAACGCCAGCCTGAGCTTCCTGCAGGACATCCAG
GAAGTGCAGGGCTACATGCTGATCGCCCACAGCCGGGTGAAGCACATCCCCCTGCAGCGGCTGAGAATCGTGCGGGGC
ACCCAGCTGTTCGAGGACAACTACGCCCTGGCCGTGCTGGACAACCGGGACCTGCAGGATAATGCCACCTCCGCCGCT
GGCAGAACACCTGAGGGCCTGCGGGAGCTGCAGCTGAGAAGCCTGACCGAGATCCTGAAGGGCGGCGTGCTGATCAGA
GGCAGCCCCCAGCTGTGCCATCAGGATATGGTGCTGTGGGAGGACGTGCTGCGGAAGAACAACCAGCTGACCCCCGTG
GACATGGACACCAACCGGTCCAGAGCCTGCCCTCCTTGCGCCCCTGCCTGCAGGGATAACCACTGCTGGGGCGCCAGC
CCAGGCGATTGCAACAGCCTGACCGGCACCATCTGCACCAGCGGCTGCGCCAGATGCAAGGGCAGACAGCCCACCGAC
TGCTGCCATGAGCAGTGTGCCGCCGGATGTACCGGCCCCAAGCACAGCGACTGCCTGGCCTGCCTGCACTTCAACCAC
AGCGGCATCTGCGAGCTGCACTGCCCCAGCCTGATCATCTACAACACCGACACCTTCGAGAGCATGCACAACCCCGAG
GGCAGATACACCTTCGGCGCCAGCTGCGTGACCACCTGCCCCTACAACTACCTGAGCACCGAAGTGGGCAGCTGCACC
CTGGTGTGCCCCCCCAACAACCAGGAAGTGACCGCCGAGGACGGCACCCAGAGATGCGAGAAGTGCAGCAAGCCCTGC
GCCAGAGTGTGTTACGGCCTGGGCATGGAACATCTGAGGGGCGCCAGGGCCATCACCAGCGACAACGTGCAGGACTTC
GTGGGCTGCAAGAAGATTTTCGGCTCCCTGGCCTTCCTGCCCGAGAGCTTCGACGGCGACCCTAGCAGCGGCATCGCC
CCCCTGAGACCAGAGCACCTGCGGGTGTTCGAGGCCCTGGAAGAGATCACCGGCTACCTGTACATCAGCGCCTGGCCC
GAGTCCTTCCGGAACCTGAGCGTGCTGCAGAACCTGCGGATCATCCGGGGCAGAGTGCTGCACGATGGCGCCTATAGC
CTCGCTCTGCAGGGACTGGGAATCAGAAGCCTGGGCCTGCGGTCTCTGCAGGAACTGGGCAGCGGACTGGCCCTGGTG
CACCGGAACGCCCGGCTGTGCTTCGTGAATACCGTGCCCTGGGCCCAGCTGTTTAGGAACCCCCACCAGGCTCTGCTG
CACAGCGGCAACCCCAGCGAGGACGAGTGCGGCCTGAAGGACTTTGTGTGCAACTCCCTGTGCGCCCACGGACACTGT
TGGGGACCTGGACCTACCCACTGCGTGAACTGCAGCCAGTTTCTGCCTGGCCAGGAATGCGTGAAAGAATGCAGAGTG
TGGAAGGGCCTGCCTCGGGAGTACGTGAGCGACAAGCGGTGCCTGCCCTGCCACAGCGAGTGCCAGCCCCAGAACAGC
ACCGAGACCTGCTACGGCAGCGAGGCCGACCAGTGTGAGGCCTGCACCCACTACAAGGACCCCCCCTTCTGCGTGGCC
AGATGCCCTAGCGGCGTGAAGCCCGACCTGAGCTACATGCCCATCTGGAAGTTCCCCGACGAGGAAGGCGCCTGCCAG
CCCTGCCCCATCAACTGCACCCACAGCTGCGCCGACCTGGACGATAGAGGCTGCCCTGCCGAG

SEQ ID NO:7: Amino Acid Sequence of CTD-derived Peptide 1 (CTD1)

NEDLGPSSPMDSTFYRSLLEDEDMGELVDAEEYLVPQQGFFSPDPTPGTGSTAHRRHRSSSARNGGGDLTLGMEPSGE
GPPRSPRAPSEGTGSDVFDGDLAVGVTKGLQSLSPQDLSPLQRYSEDPTLPLPSETDGKVAPLSCSPQPEFVNQSDVQ
PKSPLTPEGPPSPARPTGATLERAKTLSPGKNGVVKDVFTFGGAVENPEFLAPREGTASPPHPSPAFSPAFDNLFFWD
QNSSEQGPPPSNFEGTPTAENPEFLGLDVPV

SEQ ID NO:8: DNA Sequence Encoding the Amino Acid Sequence of CTD-derived Peptide 1 (CTD1)of SEQ ID NO: 7

AACGAGGATCTGGGCCCCAGCAGCCCTATGGACAGCACCTTCTACAGATCCCTGCTGGAAGATGAGGACATGGGCGAA
CTGGTGGACGCCGAGGAATACCTGGTGCCTCAGCAGGGCTTCTTCAGCCCCGATCCTACCCCTGGCACCGGCAGCACA
GCCCATCGGCGGCACAGAAGCAGTTCTGCTAGAAATGGCGGCGGAGACCTGACCCTGGGAATGGAACCTAGCGGCGAG
GGCCCTCCTAGAAGCCCTAGAGCACCTTCCGAAGGGACCGGCTCCGACGTGTTCGATGGCGATCTGGCCGTGGGCGTG

ACAAAGGGCCTGCAGTCTCTCTCTCCACAGGATCTGTCTCCACTGCAGAGATACAGCGAGGACCCCACCCTGCCTCTG
CCTAGCGAGACCGACGGCAAGGTGGCCCCTCTGAGCTGTAGCCCCCAGCCCGAGTTCGTGAACCAGAGCGACGTGCAG
CCCAAGAGCCCTCTGACCCCTGAGGGACCCCCTAGCCCTGCCAGACCTACCGGCGCCACCCTGGAAAGAGCCAAGACC
CTGAGCCCCGGCAAGAACGGCGTGGTGAAGGACGTGTTCACCTTTGGCGGAGCCGTGGAGAACCCTGAGTTCCTGGCC
CCAAGAGAGGGCACAGCCAGCCCTCCTCACCCCAGCCCAGCCTTCAGCCCTGCCTTCGACAACCTGTTCTTCTGGGAC
CAGAATTCTAGTGAACAGGGACCTCCACCCAGCAATTTCGAGGGCACCCCCACCGCCGAGAATCCCGAGTTTCTGGGC
CTGGACGTGCCCGTGTAG

SEQ ID NO:9. Amino Acid Sequence of a Peptide Composed of ECD1, TMD1, and CTD1

MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDIVRHLYQGCQVVQGNLELTYVPANASLSFLQDIQ
EVQGYMLIAHSRVKHIPLQRLRIVRGTQLFEDNYALAVLDNRDLQDNATSAAGRTPEGLRELQLRSLTEILKGGVLIR
GSPQLCHQDMVLWEDVLRKNNQLTPVDMDTNRSRACPPCAPACRDNHCWGASPGDCNSLTGTICTSGCARCKGRQPTD
CCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPSLIIYNTDTFESMHNPEGRYTFGASCVTTCPYNYLSTEVGSCT
LVCPPNNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLRGARAITSDNVQDFVGCKKIFGSLAFLPESFDGDPSSGIA
PLRPEHLRVFEALEEITGYLYISAWPESFRNLSVLQNLRIIRGRVLHDGAYSLALQGLGIRSLGLRSLQELGSGLALV
HRNARLCFVNTVPWAQLFRNPHQALLHSGNPSEDECGLKDFVCNSLCAHGHCWGPGPTHCVNCSQFLPGQECVKECRV
WKGLPREYVSDKRCLPCHSECQPQNSTETCYGSEADQCEACTHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQ
PCPINCTHSCADLDDRGCPAEQRASPLTSIVSAVVGILLVVVLGVVFGILINEDLGPSSPMDSTFYRSLLEDEDMGEL
VDAEEYLVPQQGFFSPDPTPGTGSTAHRRHRSSSARNGGGDLTLGMEPSGEGPPRSPRAPSEGTGSDVFDGDLAVGVT
KGLQSLSPQDLSPLQRYSEDPTLPLPSETDGKVAPLSCSPQPEFVNQSDVQPKSPLTPEGPPSPARPTGATLERAKTL
SPGKNGVVKDVFTFGGAVENPEFLAPREGTASPPHPSPAFSPAFDNLFFWDQNSSEQGPPPSNFEGTPTAENPEFLGL
DVPV

SEQ ID NO: 10: DNA Sequence Encoding the Amino Acid Sequence of SEQ ID NO: 9

ATGGAACTGGCCGCCCTGTGTAGATGGGGACTGCTGCTGGCCCTGCTGCCCCCTGGCGCTGCTTCCACACAGGTGTGC
ACCGGCACCGACATGAAGCTGAGACTGCCCGCCAGCCCTGAGACCCACCTGGACATCGTGCGGCACCTGTACCAGGGC
TGTCAGGTGGTGCAGGGCAACCTGGAACTGACCTACGTGCCCGCCAACGCCAGCCTGAGCTTCCTGCAGGACATCCAG
GAAGTGCAGGGCTACATGCTGATCGCCCACAGCCGGGTGAAGCACATCCCCCTGCAGCGGCTGAGAATCGTGCGGGGC
ACCCAGCTGTTCGAGGACAACTACGCCCTGGCCGTGCTGGACAACCGGGACCTGCAGGATAATGCCACCTCCGCCGCT
GGCAGAACACCTGAGGGCCTGCGGGAGCTGCAGCTGAGAAGCCTGACCGAGATCCTGAAGGGCGGCGTGCTGATCAGA
GGCAGCCCCCAGCTGTGCCATCAGGATATGGTGCTGTGGGAGGACGTGCTGCGGAAGAACAACCAGCTGACCCCCGTG
GACATGGACACCAACCGGTCCAGAGCCTGCCCTCCTTGCGCCCCTGCCTGCAGGGATAACCACTGCTGGGGCGCCAGC
CCAGGCGATTGCAACAGCCTGACCGGCACCATCTGCACCAGCGGCTGCGCCAGATGCAAGGGCAGACAGCCCACCGAC
TGCTGCCATGAGCAGTGTGCCGCCGGATGTACCGGCCCCAAGCACAGCGACTGCCTGGCCTGCCTGCACTTCAACCAC
AGCGGCATCTGCGAGCTGCACTGCCCCAGCCTGATCATCTACAACACCGACACCTTCGAGAGCATGCACAACCCCGAG
GGCAGATACACCTTCGGCGCCAGCTGCGTGACCACCTGCCCCTACAACTACCTGAGCACCGAAGTGGGCAGCTGCACC
CTGGTGTGCCCCCCCAACAACCAGGAAGTGACCGCCGAGGACGGCACCCAGAGATGCGAGAAGTGCAGCAAGCCCTGC
GCCAGAGTGTGTTACGGCCTGGGCATGGAACATCTGAGGGGCGCCAGGGCCATCACCAGCGACAACGTGCAGGACTTC

GTGGGCTGCAAGAAGATTTTCGGCTCCCTGGCCTTCCTGCCCGAGAGCTTCGACGGCGACCCTAGCAGCGGCATCGCC
CCCCTGAGACCAGAGCACCTGCGGGTGTTCGAGGCCCTGGAAGAGATCACCGGCTACCTGTACATCAGCGCCTGGCCC
GAGTCCTTCCGGAACCTGAGCGTGCTGCAGAACCTGCGGATCATCCGGGGCAGAGTGCTGCACGATGGCGCCTATAGC
CTCGCTCTGCAGGGACTGGGAATCAGAAGCCTGGGCCTGCGGTCTCTGCAGGAACTGGGCAGCGGACTGGCCCTGGTG
CACCGGAACGCCCGGCTGTGCTTCGTGAATACCGTGCCCTGGGCCCAGCTGTTTAGGAACCCCCACCAGGCTCTGCTG
CACAGCGGCAACCCCAGCGAGGACGAGTGCGGCCTGAAGGACTTTGTGTGCAACTCCCTGTGCGCCCACGGACACTGT
TGGGGACCTGGACCTACCCACTGCGTGAACTGCAGCCAGTTTCTGCCTGGCCAGGAATGCGTGAAAGAATGCAGAGTG
TGGAAGGGCCTGCCTCGGGAGTACGTGAGCGACAAGCGGTGCCTGCCCTGCCACAGCGAGTGCCAGCCCCAGAACAGC
ACCGAGACCTGCTACGGCAGCGAGGCCGACCAGTGTGAGGCCTGCACCCACTACAAGGACCCCCCCTTCTGCGTGGCC
AGATGCCCTAGCGGCGTGAAGCCCGACCTGAGCTACATGCCCATCTGGAAGTTCCCCGACGAGGAAGGCGCCTGCCAG
CCCTGCCCCATCAACTGCACCCACAGCTGCGCCGACCTGGACGATAGAGGCTGCCCTGCCGAGCAGAGAGCCAGCCCT
CTGACGTCCATCGTCTCTGCGGTGGTTGGCATTCTGCTGGTCGTGGTCTTGGGGGTGGTCTTTGGGATCCTCATCAAC
GAGGATCTGGGCCCCAGCAGCCCTATGGACAGCACCTTCTACAGATCCCTGCTGGAAGATGAGGACATGGGCGAACTG
GTGGACGCCGAGGAATACCTGGTGCCTCAGCAGGGCTTCTTCAGCCCCGATCCTACCCCTGGCACCGGCAGCACAGCC
CATCGGCGGCACAGAAGCAGTTCTGCTAGAAATGGCGGCGGAGACCTGACCCTGGGAATGGAACCTAGCGGCGAGGGC
CCTCCTAGAAGCCCTAGAGCACCTTCCGAAGGGACCGGCTCCGACGTGTTCGATGGCGATCTGGCCGTGGGCGTGACA
AAGGGCCTGCAGTCTCTCTCTCCACAGGATCTGTCTCCACTGCAGAGATACAGCGAGGACCCCACCCTGCCTCTGCCT
AGCGAGACCGACGGCAAGGTGGCCCCTCTGAGCTGTAGCCCCCAGCCCGAGTTCGTGAACCAGAGCGACGTGCAGCCC
AAGAGCCCTCTGACCCCTGAGGGACCCCCTAGCCCTGCCAGACCTACCGGCGCCACCCTGGAAAGAGCCAAGACCCTG
AGCCCCGGCAAGAACGGCGTGGTGAAGGACGTGTTCACCTTTGGCGGAGCCGTGGAGAACCCTGAGTTCCTGGCCCCA
AGAGAGGGCACAGCCAGCCCTCCTCACCCCAGCCCAGCCTTCAGCCCTGCCTTCGACAACCTGTTCTTCTGGGACCAG
AATTCTAGTGAACAGGGACCTCCACCCAGCAATTTCGAGGGCACCCCCACCGCCGAGAATCCCGAGTTTCTGGGCCTG
GACGTGCCCGTG

SEQ ID NO:11. Amino Acid Sequence of TMD-derived Peptide 1 (TMD1)

QRASPLTSIVSAVVGILLVVVLGVVFGILI

SEQ. ID NO: 12: Amino Acid Sequence of ECD-derived Peptide 1 (ECD1) without Signal Sequence

TQVCTGTDMKLRLPASPETHLDIVRHLYQGCQVVQGNLELTYVPANASLSFLQDIQEVQGYMLIAHSRVKHIPLQRLR
IVRGTQLFEDNYALAVLDNRDLQDNATSAAGRTPEGLRELQLRSLTEILKGGVLIRGSPQLCHQDMVLWEDVLRKNNQ
LTPVDMDTNRSRACPPCAPACRDNHCWGASPGDCNSLTGTICTSGCARCKGRQPTDCCHEQCAAGCTGPKHSDCLACL
HFNHSGICELHCPSLIIYNTDTFESMHNPEGRYTFGASCVTTCPYNYLSTEVGSCTLVCPPNNQEVTAEDGTQRCEKC
SKPCARVCYGLGMEHLRGARAITSDNVQDFVGCKKIFGSLAFLPESFDGDPSSGIAPLRPEHLRVFEALEEITGYLYI
SAWPESFRNLSVLQNLRIIRGRVLHDGAYSLALQGLGIRSLGLRSLQELGSGLALVHRNARLCFVNTVPWAQLFRNPH
QALLHSGNPSEDECGLKDFVCNSLCAHGHCWGPGPTHCVNCSQFLPGQECVKECRVWKGLPREYVSDKRCLPCHSECQ
PQNSTETCYGSEADQCEACTHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCADLDDRGCPAE

SEQ ID NO: 13: DNA Sequence Encoding the Amino Acid Sequence of ECD-derived Peptide 1 of SEQ ID NO: 12

ACACAGGTGTGCACCGGCACCGACATGAAGCTGAGACTGCCCGCCAGCCCTGAGACCCACCTGGACATCGTGCGGCAC
CTGTACCAGGGCTGTCAGGTGGTGCAGGGCAACCTGGAACTGACCTACGTGCCCGCCAACGCCAGCCTGAGCTTCCTG
CAGGACATCCAGGAAGTGCAGGGCTACATGCTGATCGCCCACAGCCGGGTGAAGCACATCCCCCTGCAGCGGCTGAGA
ATCGTGCGGGGCACCCAGCTGTTCGAGGACAACTACGCCCTGGCCGTGCTGGACAACCGGGACCTGCAGGATAATGCC
ACCTCCGCCGCTGGCAGAACACCTGAGGGCCTGCGGGAGCTGCAGCTGAGAAGCCTGACCGAGATCCTGAAGGGCGGC
GTGCTGATCAGAGGCAGCCCCCAGCTGTGCCATCAGGATATGGTGCTGTGGGAGGACGTGCTGCGGAAGAACAACCAG
CTGACCCCCGTGGACATGGACACCAACCGGTCCAGAGCCTGCCCTCCTTGCGCCCCTGCCTGCAGGGATAACCACTGC
TGGGGCGCCAGCCCAGGCGATTGCAACAGCCTGACCGGCACCATCTGCACCAGCGGCTGCGCCAGATGCAAGGGCAGA
CAGCCCACCGACTGCTGCCATGAGCAGTGTGCCGCCGGATGTACCGGCCCCAAGCACAGCGACTGCCTGGCCTGCCTG
CACTTCAACCACAGCGGCATCTGCGAGCTGCACTGCCCCAGCCTGATCATCTACAACACCGACACCTTCGAGAGCATG
CACAACCCCGAGGGCAGATACACCTTCGGCGCCAGCTGCGTGACCACCTGCCCCTACAACTACCTGAGCACCGAAGTG
GGCAGCTGCACCCTGGTGTGCCCCCCCAACAACCAGGAAGTGACCGCCGAGGACGGCACCCAGAGATGCGAGAAGTGC
AGCAAGCCCTGCGCCAGAGTGTGTTACGGCCTGGGCATGGAACATCTGAGGGGCGCCAGGGCCATCACCAGCGACAAC
GTGCAGGACTTCGTGGGCTGCAAGAAGATTTTCGGCTCCCTGGCCTTCCTGCCCGAGAGCTTCGACGGCGACCCTAGC
AGCGGCATCGCCCCCCTGAGACCAGAGCACCTGCGGGTGTTCGAGGCCCTGGAAGAGATCACCGGCTACCTGTACATC
AGCGCCTGGCCCGAGTCCTTCCGGAACCTGAGCGTGCTGCAGAACCTGCGGATCATCCGGGGCAGAGTGCTGCACGAT
GGCGCCTATAGCCTCGCTCTGCAGGGACTGGGAATCAGAAGCCTGGGCCTGCGGTCTCTGCAGGAACTGGGCAGCGGA
CTGGCCCTGGTGCACCGGAACGCCCGGCTGTGCTTCGTGAATACCGTGCCCTGGGCCCAGCTGTTTAGGAACCCCCAC
CAGGCTCTGCTGCACAGCGGCAACCCCAGCGAGGACGAGTGCGGCCTGAAGGACTTTGTGTGCAACTCCCTGTGCGCC
CACGGACACTGTTGGGGACCTGGACCTACCCACTGCGTGAACTGCAGCCAGTTTCTGCCTGGCCAGGAATGCGTGAAA
GAATGCAGAGTGTGGAAGGGCCTGCCTCGGGAGTACGTGAGCGACAAGCGGTGCCTGCCCTGCCACAGCGAGTGCCAG
CCCCAGAACAGCACCGAGACCTGCTACGGCAGCGAGGCCGACCAGTGTGAGGCCTGCACCCACTACAAGGACCCCCCC
TTCTGCGTGGCCAGATGCCCTAGCGGCGTGAAGCCCGACCTGAGCTACATGCCCATCTGGAAGTTCCCCGACGAGGAA
GGCGCCTGCCAGCCCTGCCCCATCAACTGCACCCACAGCTGCGCCGACCTGGACGATAGAGGCTGCCCTGCCGAG

SEQ ID NO:14: Amino Acid Sequence of ECD-derived Peptide 2 (ECD2)

MASELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFLQD
IQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLDSVAPAAGATPGGLQELQLRSLTEILKGGVL
IRRSPQLCHQDTVLWEDVFRKNNQLALVLMDTNRSRACHPCAPMCKANHCWGESSQDCQTLTRTICTSACARCKAPLP
TDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGS
CTLVCPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREARAITSANVQDFVGCKKIFGSLAFLPESFDGDPASG
TAPLQPEQLQVFETLEEITGYLYISAWPDSFPNLSVFQNLRVIRGRILHNGAYSLTLQGLGISWLGLRSLQELGSGLA
LVHRNARLCFVHTVPWDQLFRNPHQALLHSGNRPEEDCVGEGFVCYSLCAHGHCWGPGPTQCVNCSHFLRGQECVEEC
RVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGA
CQPCPINCTHSCVDLDDKGCPAE

SEQ ID NO:15: DNA Sequence Encoding the Amino Acid Sequence of ECD-derived Peptide 2 (ECD2) of SEQ ID NO: 14

ATGGCTAGCGAGCTGGCCGCCCTGTGTAGATGGGGACTGCTGCTGGCTCTGCTGCCTCCTGGAGCCGCTTCTACACAG
GTCTGCACCGGCACCGACATGAAGCTGAGACTGCCCGCCAGCCCCGAGACACACCTGGACATGCTGCGGCACCTGTAC
CAGGGCTGCCAGGTGGTCCAGGGGAATCTGGAACTGACCTACCTGCCCACCAACGCCAGCCTGAGCTTCCTGCAGGAC

ATCCAGGAAGTGCAGGGCTACGTCCTGATCGCCCACAACCAGGTCCGCCAGGTGCCCCTGCAGCGGCTGAGAATCGTG
CGGGGCACCCAGCTGTTCGAGGACAACTACGCCCTGGCCGTGCTGGACAACGGCGACCCTCTGGATAGCGTGGCCCCT
GCTGCTGGGGCTACACCTGGCGGACTGCAGGAACTGCAGCTGCGGAGCCTGACCGAGATCCTGAAGGGCGGCGTGCTG
ATCAGGCGGAGCCCTCAGCTGTGCCACCAGGACACCGTGCTGTGGGAGGACGTGTTCCGGAAGAACAACCAGCTGGCC
CTCGTGCTGATGGACACCAACAGAAGCCGGGCCTGCCACCCCTGCGCCCCCATGTGCAAGGCCAATCACTGCTGGGGA
GAGAGCAGCCAGGACTGCCAGACCCTGACCCGGACCATCTGCACCAGCGCCTGCGCCAGATGCAAGGCCCCCCTGCCT
ACCGACTGCTGCCACGAACAGTGCGCCGCTGGCTGCACCGGCCCCAAGCACAGCGATTGCCTGGCCTGCCTGCACTTC
AACCACAGCGGCATCTGCGAGCTGCACTGCCCTGCCCTGGTGACATACAACACCGACACCTTCGAGAGCATGCCCAAC
CCCGAGGGCCGGTACACCTTCGGCGCCAGCTGTGTGACCGCCTGCCCCTACAACTACCTGAGCACCGACGTGGGCAGC
TGCACCCTGGTGTGCCCCCTGCACAACCAGGAAGTGACCGCCGAGGACGGCACCCAGAGATGCGAGAAGTGCAGCAAG
CCTTGCGCCAGAGTGTGCTACGGCCTGGGCATGGAACACCTGAGAGAGGCCAGAGCCATCACCAGCGCCAACGTGCAG
GACTTCGTGGGCTGCAAGAAGATTTTCGGCTCCCTGGCCTTCCTGCCCGAGAGCTTCGACGGCGATCCTGCCTCTGGC
ACCGCCCCTCTGCAGCCTGAGCAGCTGCAGGTCTTCGAGACACTGGAAGAGATCACCGGCTACCTGTACATCAGCGCC
TGGCCCGACAGCTTCCCCAACCTGAGCGTGTTCCAGAACCTGAGAGTGATCCGGGGCAGAATCCTGCACAACGGCGCC
TACAGCCTGACCCTGCAGGGCCTGGGAATCAGCTGGCTGGGCCTGCGGAGCCTGCAGGAACTGGGATCTGGCCTGGCT
CTGGTGCACCGGAACGCCCGGCTGTGCTTCGTGCACACCGTGCCCTGGGACCAGCTGTTCAGAAACCCCCACCAGGCT
CTGCTGCACAGCGGCAACCGGCCCGAAGAGGATTGCGTGGGCGAGGGCTTCGTGTGCTACTCCCTGTGCGCCCACGGC
CACTGTTGGGGACCTGGCCCTACCCAGTGCGTGAACTGCAGCCACTTCCTGCGGGGCCAAGAATGCGTGGAAGAGTGC
CGGGTGCTGCAGGGACTGCCCCGGGAATACGTGAACGCCAGACACTGCCTGCCTTGCCACCCCGAGTGCCAGCCCCAG
AATGGCAGCGTGACCTGCTTCGGACCCGAGGCCGATCAGTGTGTGGCCTGCGCCCACTACAAGGACCCCCCATTCTGC
GTGGCCAGATGCCCCAGCGGCGTGAAGCCCGACCTGAGCTACATGCCCATCTGGAAGTTCCCCGACGAGGAAGGCGCC
TGCCAGCCTTGCCCCATCAACTGCACCCACAGCTGCGTGGACCTGGACGACAAGGGCTGCCCTGCCGAG

SEQ ID NO: 16: Amino Acid Sequence of TMD-derived Peptide 2 (TMD2)

QRASPLTSIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRR

SEQ ID NO: 17: DNA Sequence Encoding Amino Acid Sequence of TMD-derived Peptide 2 (TMD2) of SEQ ID NO: 16

CAGAGAGCCAGCCCCCTGACCAGCATCATCAGCGCCGTGGTGGGAATCCTGCTGGTGGTGGTGCTGGGCGTGGTGTTC
GGCATCCTGATCAAGCGGCGGCAGCAGAAGATCCGGAAGTACACCATGCGGCGG

SEQ ID NO: 18: Amino Acid Sequence of a Peptide Composed of ECD2 and CTD2

MASELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFLQD
IQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLDSVAPAAGATPGGLQELQLRSLTEILKGGVL
IRRSPQLCHQDTVLWEDVFRKNNQLALVLMDTNRSRACHPCAPMCKANHCWGESSQDCQTLTRTICTSACARCKAPLP
TDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGS
CTLVCPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREARAITSANVQDFVGCKKIFGSLAFLPESFDGDPASG
TAPLQPEQLQVFETLEEITGYLYISAWPDSFPNLSVFQNLRVIRGRILHNGAYSLTLQGLGISWLGLRSLQELGSGLA
LVHRNARLCFVHTVPWDQLFRNPHQALLHSGNRPEEDCVGEGFVCYSLCAHGHCWGPGPTQCVNCSHFLRGQECVEEC
RVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGA

CQPCPINCTHSCVDLDDKGCPAENEDLGPSSPMDSTFYRSLLEDEDMGELVDAEEYLVPQQGFFCPDPTPGTGSTAHR
RHRSSSARNGGGDLTLGMEPSGEGPPRSPRAPSEGTGSDVFDGDLAVGVTKGLQSLSPQDLSPLQRYSEDPTLPLPSE
TDGKVAPLSCSPQPEFVNQSDVQPKSPLTPEGPPSPARPTGATLERAKTLSPGKNGVVKDVFTFGGAVENPEFLAPRE
GTASPPHPSPAFSPAFDNLFFWDQNSSEQGPPPSNFEGTPTAENPEFLGLDVPV

SEQ ID NO: 19: DNA Sequence Encoding the Amino Acid Sequence of SEQ ID NO: 18

ATGGCTAGCGAGCTGGCCGCCCTGTGTAGATGGGGACTGCTGCTGGCTCTGCTGCCTCCTGGAGCCGCTTCTACACAG
GTCTGCACCGGCACCGACATGAAGCTGAGACTGCCCGCCAGCCCCGAGACACACCTGGACATGCTGCGGCACCTGTAC
CAGGGCTGCCAGGTGGTCCAGGGGAATCTGGAACTGACCTACCTGCCCACCAACGCCAGCCTGAGCTTCCTGCAGGAC
ATCCAGGAAGTGCAGGGCTACGTCCTGATCGCCCACAACCAGGTCCGCCAGGTGCCCCTGCAGCGGCTGAGAATCGTG
CGGGGCACCCAGCTGTTCGAGGACAACTACGCCCTGGCCGTGCTGGACAACGGCGACCCTCTGGATAGCGTGGCCCCT
GCTGCTGGGGCTACACCTGGCGGACTGCAGGAACTGCAGCTGCGGAGCCTGACCGAGATCCTGAAGGGCGGCGTGCTG
ATCAGGCGGAGCCCTCAGCTGTGCCACCAGGACACCGTGCTGTGGGAGGACGTGTTCCGGAAGAACAACCAGCTGGCC
CTCGTGCTGATGGACACCAACAGAAGCCGGGCCTGCCACCCCTGCGCCCCCATGTGCAAGGCCAATCACTGCTGGGGA
GAGAGCAGCCAGGACTGCCAGACCCTGACCCGGACCATCTGCACCAGCGCCTGCGCCAGATGCAAGGCCCCCCTGCCT
ACCGACTGCTGCCACGAACAGTGCGCCGCTGGCTGCACCGGCCCCAAGCACAGCGATTGCCTGGCCTGCCTGCACTTC
AACCACAGCGGCATCTGCGAGCTGCACTGCCCTGCCCTGGTGACATACAACACCGACACCTTCGAGAGCATGCCCAAC
CCCGAGGGCCGGTACACCTTCGGCGCCAGCTGTGTGACCGCCTGCCCCTACAACTACCTGAGCACCGACGTGGGCAGC
TGCACCCTGGTGTGCCCCCTGCACAACCAGGAAGTGACCGCCGAGGACGGCACCCAGAGATGCGAGAAGTGCAGCAAG
CCTTGCGCCAGAGTGTGCTACGGCCTGGGCATGGAACACCTGAGAGAGGCCAGAGCCATCACCAGCGCCAACGTGCAG
GACTTCGTGGGCTGCAAGAAGATTTTCGGCTCCCTGGCCTTCCTGCCCGAGAGCTTCGACGGCGATCCTGCCTCTGGC
ACCGCCCCTCTGCAGCCTGAGCAGCTGCAGGTCTTCGAGACACTGGAAGAGATCACCGGCTACCTGTACATCAGCGCC
TGGCCCGACAGCTTCCCCAACCTGAGCGTGTTCCAGAACCTGAGAGTGATCCGGGGCAGAATCCTGCACAACGGCGCC
TACAGCCTGACCCTGCAGGGCCTGGGAATCAGCTGGCTGGGCCTGCGGAGCCTGCAGGAACTGGGATCTGGCCTGGCT
CTGGTGCACCGGAACGCCCGGCTGTGCTTCGTGCACACCGTGCCCTGGGACCAGCTGTTCAGAAACCCCCACCAGGCT
CTGCTGCACAGCGGCAACCGGCCCGAAGAGGATTGCGTGGGCGAGGGCTTCGTGTGCTACTCCCTGTGCGCCCACGGC
CACTGTTGGGGACCTGGCCCTACCCAGTGCGTGAACTGCAGCCACTTCCTGCGGGGCCAAGAATGCGTGGAAGAGTGC
CGGGTGCTGCAGGGACTGCCCCGGGAATACGTGAACGCCAGACACTGCCTGCCTTGCCACCCCGAGTGCCAGCCCCAG
AATGGCAGCGTGACCTGCTTCGGACCCGAGGCCGATCAGTGTGTGGCCTGCGCCCACTACAAGGACCCCCCATTCTGC
GTGGCCAGATGCCCCAGCGGCGTGAAGCCCGACCTGAGCTACATGCCCATCTGGAAGTTCCCCGACGAGGAAGGCGCC
TGCCAGCCTTGCCCCATCAACTGCACCCACAGCTGCGTGGACCTGGACGACAAGGGCTGCCCTGCCGAGAACGAGGAC
CTGGGCCCCTCTAGCCCCATGGACAGCACCTTCTACCGGTCCCTGCTGGAAGATGAGGACATGGGCGAGCTGGTGGAC
GCCGAGGAATACCTGGTGCCTCAGCAGGGCTTCTTCTGCCCCGACCCTACCCCTGGCACCGGCTCTACCGCCCACAGA
CGGCACAGAAGCAGCAGCGCCAGAAACGGCGGAGGCGACCTGACCCTGGGAATGGAACCTAGCGGCGAGGGACCTCCC
AGAAGCCCTAGAGCCCCTAGCGAGGGCACCGGCAGCGACGTGTTCGATGGCGATCTGGCCGTGGGCGTGACCAAGGGA
CTGCAGAGCCTGAGCCCCCAGGACCTGTCCCCCCTGCAGAGATACAGCGAGGACCCCACCCTGCCCCTGCCCAGCGAG
ACAGATGGCAAGGTGGCCCCCCTGAGCTGCAGCCCTCAGCCCGAGTTCGTGAACCAGAGCGACGTGCAGCCCAAGTCC
CCCCTGACACCCGAGGGACCTCCAAGCCCTGCCAGACCTACCGGCGCCACCCTGGAAAGAGCCAAGACCCTGAGCCCC
GGCAAGAACGGCGTGGTGAAAGACGTGTTCACCTTCGGAGGCGCCGTGGAAAACCCCGAGTTCCTGGCCCCCAGAGAG
GGCACAGCCAGCCCTCCACACCCCAGCCCAGCCTTCTCCCCCGCCTTCGACAACCTGTTCTTCTGGGACCAGAACAGC

AGCGAGCAGGGCCCACCCCCCAGCAATTTCGAGGGCACCCCCACCGCCGAGAATCCTGAGTTCCTGGGCCTGGACGTG
CCCGTGTGA

SEQ ID NO: 20: Amino Acid Sequence of RaDHER2-2

MASELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFLQD
IQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLDSVAPAAGATPGGLQELQLRSLTEILKGGVL
IRRSPQLCHQDTVLWEDVFRKNNQLALVLMDTNRSRACHPCAPMCKANHCWGESSQDCQTLTRTICTSACARCKAPLP
TDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGS
CTLVCPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREARAITSANVQDFVGCKKIFGSLAFLPESFDGDPASG
TAPLQPEQLQVFETLEEITGYLYISAWPDSFPNLSVFQNLRVIRGRILHNGAYSLTLQGLGISWLGLRSLQELGSGLA
LVHRNARLCFVHTVPWDQLFRNPHQALLHSGNRPEEDCVGEGFVCYSLCAHGHCWGPGPTQCVNCSHFLRGQECVEEC
RVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGA
CQPCPINCTHSCVDLDDKGCPAEQRASPLTSIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRRNEDLGPSSPMD
STFYRSLLEDEDMGELVDAEEYLVPQQGFFCPDPTPGTGSTAHRRHRSSSARNGGGDLTLGMEPSGEGPPRSPRAPSE
GTGSDVFDGDLAVGVTKGLQSLSPQDLSPLQRYSEDPTLPLPSETDGKVAPLSCSPQPEFVNQSDVQPKSPLTPEGPP
SPARPTGATLERAKTLSPGKNGVVKDVFTFGGAVENPEFLAPREGTASPPHPSPAFSPAFDNLFFWDQNSSEQGPPPS
NFEGTPTAENPEFLGLDVPV

SEQ ID NO: 21: DNA Sequence Encoding the Amino Acid Sequence of RaDHER2-2 of SEQ ID NO: 20

ATGGCTAGCGAGCTGGCCGCCCTGTGTAGATGGGGACTGCTGCTGGCTCTGCTGCCTCCTGGAGCCGCTTCTACACAG
GTCTGCACCGGCACCGACATGAAGCTGAGACTGCCCGCCAGCCCCGAGACACACCTGGACATGCTGCGGCACCTGTAC
CAGGGCTGCCAGGTGGTCCAGGGGAATCTGGAACTGACCTACCTGCCCACCAACGCCAGCCTGAGCTTCCTGCAGGAC
ATCCAGGAAGTGCAGGGCTACGTCCTGATCGCCCACAACCAGGTCCGCCAGGTGCCCCTGCAGCGGCTGAGAATCGTG
CGGGGCACCCAGCTGTTCGAGGACAACTACGCCCTGGCCGTGCTGGACAACGGCGACCCTCTGGATAGCGTGGCCCCT
GCTGCTGGGGCTACACCTGGCGGACTGCAGGAACTGCAGCTGCGGAGCCTGACCGAGATCCTGAAGGGCGGCGTGCTG
ATCAGGCGGAGCCCTCAGCTGTGCCACCAGGACACCGTGCTGTGGGAGGACGTGTTCCGGAAGAACAACCAGCTGGCC
CTCGTGCTGATGGACACCAACAGAAGCCGGGCCTGCCACCCCTGCGCCCCCATGTGCAAGGCCAATCACTGCTGGGGA
GAGAGCAGCCAGGACTGCCAGACCCTGACCCGGACCATCTGCACCAGCGCCTGCGCCAGATGCAAGGCCCCCCTGCCT
ACCGACTGCTGCCACGAACAGTGCGCCGCTGGCTGCACCGGCCCCAAGCACAGCGATTGCCTGGCCTGCCTGCACTTC
AACCACAGCGGCATCTGCGAGCTGCACTGCCCTGCCCTGGTGACATACAACACCGACACCTTCGAGAGCATGCCCAAC
CCCGAGGGCCGGTACACCTTCGGCGCCAGCTGTGTGACCGCCTGCCCCTACAACTACCTGAGCACCGACGTGGGCAGC
TGCACCCTGGTGTGCCCCCTGCACAACCAGGAAGTGACCGCCGAGGACGGCACCCAGAGATGCGAGAAGTGCAGCAAG
CCTTGCGCCAGAGTGTGCTACGGCCTGGGCATGGAACACCTGAGAGAGGCCAGAGCCATCACCAGCGCCAACGTGCAG
GACTTCGTGGGCTGCAAGAAGATTTTCGGCTCCCTGGCCTTCCTGCCCGAGAGCTTCGACGGCGATCCTGCCTCTGGC
ACCGCCCCTCTGCAGCCTGAGCAGCTGCAGGTCTTCGAGACACTGGAAGAGATCACCGGCTACCTGTACATCAGCGCC
TGGCCCGACAGCTTCCCCAACCTGAGCGTGTTCCAGAACCTGAGAGTGATCCGGGGCAGAATCCTGCACAACGGCGCC
TACAGCCTGACCCTGCAGGGCCTGGGAATCAGCTGGCTGGGCCTGCGGAGCCTGCAGGAACTGGGATCTGGCCTGGCT
CTGGTGCACCGGAACGCCCGGCTGTGCTTCGTGCACACCGTGCCCTGGGACCAGCTGTTCAGAAACCCCCACCAGGCT
CTGCTGCACAGCGGCAACCGGCCCGAAGAGGATTGCGTGGGCGAGGGCTTCGTGTGCTACTCCCTGTGCGCCCACGGC
CACTGTTGGGGACCTGGCCCTACCCAGTGCGTGAACTGCAGCCACTTCCTGCGGGGCCAAGAATGCGTGGAAGAGTGC

CGGGTGCTGCAGGGACTGCCCCGGGAATACGTGAACGCCAGACACTGCCTGCCTTGCCACCCCGAGTGCCAGCCCCAG
AATGGCAGCGTGACCTGCTTCGGACCCGAGGCCGATCAGTGTGTGGCCTGCGCCCACTACAAGGACCCCCCATTCTGC
GTGGCCAGATGCCCCAGCGGCGTGAAGCCCGACCTGAGCTACATGCCCATCTGGAAGTTCCCCGACGAGGAAGGCGCC
TGCCAGCCTTGCCCCATCAACTGCACCCACAGCTGCGTGGACCTGGACGACAAGGGCTGCCCTGCCGAGCAGAGAGCC
AGCCCCCTGACCAGCATCATCAGCGCCGTGGTGGGAATCCTGCTGGTGGTGGTGCTGGGCGTGGTGTTCGGCATCCTG
ATCAAGCGGCGGCAGCAGAAGATCCGGAAGTACACCATGCGGCGGAACGAGGACCTGGGCCCCTCTAGCCCCATGGAC
AGCACCTTCTACCGGTCCCTGCTGGAAGATGAGGACATGGGCGAGCTGGTGGACGCCGAGGAATACCTGGTGCCTCAG
CAGGGCTTCTTCTGCCCCGACCCTACCCCTGGCACCGGCTCTACCGCCCACAGACGGCACAGAAGCAGCAGCGCCAGA
AACGGCGGAGGCGACCTGACCCTGGGAATGGAACCTAGCGGCGAGGGACCTCCCAGAAGCCCTAGAGCCCCTAGCGAG
GGCACCGGCAGCGACGTGTTCGATGGCGATCTGGCCGTGGGCGTGACCAAGGGACTGCAGAGCCTGAGCCCCCAGGAC
CTGTCCCCCCTGCAGAGATACAGCGAGGACCCCACCCTGCCCCTGCCCAGCGAGACAGATGGCAAGGTGGCCCCCCTG
AGCTGCAGCCCTCAGCCCGAGTTCGTGAACCAGAGCGACGTGCAGCCCAAGTCCCCCCTGACACCCGAGGGACCTCCA
AGCCCTGCCAGACCTACCGGCGCCACCCTGGAAAGAGCCAAGACCCTGAGCCCCGGCAAGAACGGCGTGGTGAAAGAC
GTGTTCACCTTCGGAGGCGCCGTGGAAAACCCCGAGTTCCTGGCCCCCAGAGAGGGCACAGCCAGCCCTCCACACCCC
AGCCCAGCCTTCTCCCCCGCCTTCGACAACCTGTTCTTCTGGGACCAGAACAGCAGCGAGCAGGGCCCACCCCCCAGC
AATTTCGAGGGCACCCCCACCGCCGAGAATCCTGAGTTCCTGGGCCTGGACGTGCCCGTGTGA

SEQ ID NO: 22: Amino Acid Sequence of CTD-derived Peptide 2 (CTD2)

NEDLGPSSPMDSTFYRSLLEDEDMGELVDAEEYLVPQQGFFCPDPTPGTGSTAHRRHRSSSARNGGGDLTLGMEPSGE
GPPRSPRAPSEGTGSDVFDGDLAVGVTKGLQSLSPQDLSPLQRYSEDPTLPLPSETDGKVAPLSCSPQPEFVNQSDVQ
PKSPLTPEGPPSPARPTGATLERAKTLSPGKNGVVKDVFTFGGAVENPEFLAPREGTASPPHPSPAFSPAFDNLFFWD
QNSSEQGPPPSNFEGTPTAENPEFLGLDVPV

SEQ ID NO: 23: DNA Sequence Encoding the Amino Acid Sequence of CTD-derived Peptide 2 (CTD2) of SEQ ID NO: 22

AACGAGGACCTGGGCCCCTCTAGCCCCATGGACAGCACCTTCTACCGGTCCCTGCTGGAAGATGAGGACATGGGCGAG
CTGGTGGACGCCGAGGAATACCTGGTGCCTCAGCAGGGCTTCTTCTGCCCCGACCCTACCCCTGGCACCGGCTCTACC
GCCCACAGACGGCACAGAAGCAGCAGCGCCAGAAACGGCGGAGGCGACCTGACCCTGGGAATGGAACCTAGCGGCGAG
GGACCTCCCAGAAGCCCTAGAGCCCCTAGCGAGGGCACCGGCAGCGACGTGTTCGATGGCGATCTGGCCGTGGGCGTG
ACCAAGGGACTGCAGAGCCTGAGCCCCCAGGACCTGTCCCCCCTGCAGAGATACAGCGAGGACCCCACCCTGCCCCTG
CCCAGCGAGACAGATGGCAAGGTGGCCCCCCTGAGCTGCAGCCCTCAGCCCGAGTTCGTGAACCAGAGCGACGTGCAG
CCCAAGTCCCCCCTGACACCCGAGGGACCTCCAAGCCCTGCCAGACCTACCGGCGCCACCCTGGAAAGAGCCAAGACC
CTGAGCCCCGGCAAGAACGGCGTGGTGAAAGACGTGTTCACCTTCGGAGGCGCCGTGGAAAACCCCGAGTTCCTGGCC
CCCAGAGAGGGCACAGCCAGCCCTCCACACCCCAGCCCAGCCTTCTCCCCCGCCTTCGACAACCTGTTCTTCTGGGAC
CAGAACAGCAGCGAGCAGGGCCCACCCCCCAGCAATTTCGAGGGCACCCCCACCGCCGAGAATCCTGAGTTCCTGGGC
CTGGACGTGCCCGTGTGA

SEQ ID NO:24: **DNA Sequence Encoding the** Amino Acid Sequence of TMD-derived Peptide 1 (TMD1) of **SEQ ID NO: 11**

CAGAGAGCCAGCCCCCTGACCAGCATCATCAGCGCCGTGGTGGGAATCCTGCTGGTGGTGGTGCTGGGCGTGGTGTTC
GGCATCCTGATC

G. Examples

The following examples are provided to illustrate certain embodiments of the invention. They should not be construed to limit the scope of the invention in any way. From the above discussion and these examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usage and conditions.

Example 1. Design of Immunogenic HER-2 Peptides

1). RaDHER2-1

The Immunogenic HER-2 peptide RaDHER2-1 comprises an ECD-derived peptide joined to a CTD-derived peptide. The amino acid sequence of RaDHER2-1 is set forth in SEQ ID No: 3. SEQ ID NO:3 also includes the amino acid sequence of the signal sequence (amino acid residues 1-22). RaDHER2-1 was designed by aligning the human HER-2 protein sequence with the orthologous sequences of rhesus monkey, horse, dog, cat, rat, and mouse using ClustalW and introducing certain point mutations into human HER2 extracellular domain (ECD) and cytoplasmic domain (CTD) based on amino acid substitutions observed in the orthologs. One or more amino acids were selected from each of the six orthologous sequences indicated above. These substitutions were selected in positions outside of the conserved T cell epitope sequences identified in the application above and outside of the signal sequence. This resulted in 126 point mutations in the 645 amino acids of the ECD domain. Likewise, point substitutions were produced in the CT domain, along with several tyrosine to phenylalanine mutations (to avoid phosphorylation in the modified CT sequence due to safety consideration), resulting in 62 mutations out of 265 amino acid residues. The final designed HER-2 peptide, which is referred to as “RaDHER2-1” in this application, consists of the modified ECD domain containing 126 point mutations fused to the modified CT domain containing 62 point mutations. For expressing the full RaDHER2-1 sequence, the DNA sequences that encode the ECD-derived peptide and the CTD-derived peptide sequences described above were fused to form a single transcript and cloned into suitable vectors. In addition, codons for a restriction enzyme site (amino acid residues alanine and serine) were inserted into the RaDHER2-1 DNA sequence between amino acid residues 1 (methionine) and 2 of the RaDHER2-1 peptide. The

RaDHER2-1 DNA sequence containing the restriction enzyme site was used in the experiments described below.

2) RaDHER2-2

The Immunogenic HER-2 peptide RaDHER2-2 is a fusion protein that comprises an ECD-derived peptide, a TMD-derived peptide, and a CTD-derived peptide. The amino acid sequence of RaDHER2-1 is set forth in SEQ ID No: 20. In addition, SEQ ID NO: 20 includes the amino acid sequence of the signal sequence (amino acid residues 1-24). The signal sequence further includes a restriction enzyme site (amino acid residues alanine at position 2 and serine at position 3). In the design of RaDHER2-2, in addition to the HER-2 sequences of rhesus monkey, horse, dog, cat, rat, and mouse, the orthologous sequences of chimp, hamster, and opossum were also aligned with the human HER-2 protein, The ECD-derived peptide of contains 52 amino acid substitutions. The selection of amino acid substitutions is based on the same principle as for the design of RadHER2-1. The CTD-derived peptide includes one additional amino acid change from the CTD-derived peptide of RaDHER2-1.

Example 2. Cloning, Expression and PMED Formulations of RaDHER2-1 and RadHER2-2

2a. Cloning of RaDHER2-1 and RadHER2-2 into PJV7563 vector: The immunogenic HER-2 peptide amino acid sequence was codon optimized for optimal expression in mammalian cells and cloned into a vector (PJV7563) that was suitable for in vivo testing in animals (Figure 1). Both strands of the RaDHER2 DNA in the PJV7563 vector were completely sequenced to confirm the designed sequence.

2b. Plasmid DNA production: A large scale plasmid DNA (Qiagen/CsCl) was produced from a sequence confirmed clone. The quality of the plasmid DNA was confirmed by high 260/280 ratio, high super coiled/nicked DNA ratio, low endotoxin levels (< 10U/mg DNA) and negative bio burden.

2c. Expression of the RaDHER2-1 and RadHER2-2 construct in mammalian cells: The protein expression of RaDHER2 was determined by Western blot analysis of cell extracts from 293FS cells expressing HER-2 peptide by Lipofectamine 2000 mediated transient transfection with PJV7563 vector containing the RaDHER2 as described by the manufacturer (Invitrogen). Five days after the transfection both the media and the cells were harvested for expression analysis by Western. Commercially available anti-HER2 antibody (Thermo Scientific, clone 3B5) was used for the detection

of HER-2 peptide. The expression of HER-2 peptide was detected in both the media and the cell lysate, indicating that RaDHER2 protein was expressed.

2d. Formulations of RaDHER2-1 and RadHER2-2 (gold particles, and ND10): Particle Mediated Epidermal Delivery technology (PMED), is a needle-free method of administering vaccines to animals or to patients. The PMED system involves the precipitation of DNA onto microscopic gold particles that are then propelled by helium gas into the epidermis. The DNA-coated gold particles are delivered into the antigen-presenting cells (APCs) of the epidermis, and once inside the nuclei of the APCs, the DNA elutes off the gold and becomes transcriptionally active, producing encoded protein. This protein is then presented by the APCs to the lymphocytes to induce a T-cell-mediated immune response. The ND10 delivery device uses pressurized helium from an internal cylinder to accelerate gold particles of 1–3 μm diameter coated with DNA into the epidermis. ND10 devices used in *in vivo* studies were formulated to contain 2 μg of the HER-2 NDA per 1mg of gold particles. Control plasmids were also formulated similarly for PMED vaccination (Sharpe, M. Lynch, D. Topham, S. Major, D. Wood, J and Loudon, P. Protection of mice from H5N1 influenza challenge by prophylactic DNA vaccination using particle mediated epidermal delivery. Vaccine, 2007, 25(34): 6392-98: Roberts LK, Barr LJ, Fuller DH, McMahon CW, Leese PT, Jones S. Clinical safety and efficacy of a powdered Hepatitis B nucleic acid vaccine delivered to the epidermis by a commercial prototype device. Vaccine, 2005; 23(40):4867–78.).

2e. PMED vaccination: Eight to ten weeks old mice were immunized with PMED control or HER2 containing antigen in a prime/boost format and the immune response was measured 7 days after the last boost in the splenocytes of mice unless otherwise indicated.

Example 3. Immunogenicity studies with RaDHER2-1 and RadHER2-2: The immunogenicity of RaDHER2-1 and RadHER2-2 evaluated in different in *vivo* and *in vitro* models: (a) Balb/c mice for breaking T cell tolerance (b) HLA.A2 transgenic mice and (c) *in vitro* human PBMC assay for the processing and presentation of the HER-2 in the HLA-A2 context. Amino acid sequences of certain peptides that were used in the studies are provided below:

HBVc Kd/C, p87:	SYVNTNMGL (SEQ ID NO: 25)
Rat HER2, p66:	TYVPANASL (SEQ ID NO: 26)
Rat HER2, p169:	DMVLWKDVFRKNNQL (SEQ ID NO: 27)
MouseHER2, p63:	TYLPANASL (SEQ ID NO: 28)

hHBV, p18 : FLPSDFFPSV (SEQ ID NO: 29)
Human HER2, p5: ALCRWGLL (SEQ ID NO: 30)
Human HER2, p48: HLYQGCQVV (SEQ ID NO: 31)
Human HER2, p98: RLRIVRGTQLFEDNYAL (SEQ ID NO: 32)
Human HER2, p106: QLFEDNYAL (SEQ ID NO: 33)
Human HER2, p369: KIFGSLAFL (SEQ ID NO: 34)
Human HER2, p435: ILHNGAYSL (SEQ ID NO: 35)

3a. Immunogenicity studies in BALB/c mice: BALB/c mice were vaccinated twice in a two week interval with RaDHER2-1 DNA formulated (2µg plasmid DNA/immunization) for PMED and the T cell immune response was measured 7 days after the last immunization by IFNγ ELISPOT assay. Human HER2 and rat HER2 vaccines were included as controls. The frequency of antigen specific IFN-γ secreting T cells in the spleen was assessed against H-2d restricted targets cells (P815) that were either pulsed with rat HER2 peptides or an irrelevant peptide (derived from HBVc antigen) and TUBO cells that endogenously express rat HER2 antigen. Specific T cell responses to rat and mouse HER2 peptide pulsed target cells and TUBO cells were observed in mice immunized with rat, RaDHER2 and human HER2 antigens but not to P815 cells pulsed with irrelevant HBVc peptide. Results are presented in Table 1. Data plot is normalized to the number of cells that secrete IFNγ in 1e6 splenocytes.

Table 1. Tolerance to self HER-2 antigen is broken in BALB/c mice vaccinated with xenogeneic HER2 vaccines.

Peptide pulsed targets and rat HER-2+ tumor cells	IFNγ SFC/1e6 splenocytes		
	Rat HER2	Human HER2	RaDHER2-1
Splenocyte only	1(1)	5 (6)	2 (2)
HBVc Kd /C87-95	2 (2)	2 (2)	3 (1)
Rat HER-2 p66	829 (3)	55 (4)	831 (26)
Rat HER-2 p65	32 (8)	5 (3)	9 (1)
Rat HER-2 p169	106 (10)	194 (26)	11 (1)
Rat HER-2 p393	160 (15)	14 (4)	87 (11)
Rat HER-2 p624	449 (47)	10 (2)	439 (31)
Mouse HER-2 p63	243 (16)	57 (12)	133 (30)
TUBO	291 (29)	8 (6)	389 (70)

( ) = SD

All three xenogeneic vaccines, rat HER2, human HER2 and RaDHER2-1 vaccines induces specific T responses to both rat and mouse HER2 peptides. The results indicate that RaDHER2-1, as well as the rat and human HER2 antigens, broke immune tolerance to mouse HER2 as demonstrated by the recognition of target cells pulsed with mouse HER2 p63 peptide.

3b. Immunogenicity studies in HLA A2/DR1 mice: A human HLA transgenic model was used to evaluate the processing and presentation of the RaDHER2-1 and RaDHER2-2 peptide. The model is the HLA A2/DR1 mice from the Pasteur Institute (Paris, France), HLA A2/DR mice are KO for murine β-2-microglobulin and do not express functional H-2b molecules; therefore this model would represent the processing and presentation of antigen in the human MHC I system (Pajot, A., M.-L. Michel, N. Faxilleau, V. Pancre, C. Auriault, D.M. Ojcius, F.A. Lemonnier, and Y.-C. Lone. A mouse model of human adaptive immune functions: HLA-A2.1-/HLA-DR1-transgenic H-2 class I-/class II-knockout mice. Eur. J. Immunol. 2004, 34:3060-69.). HLA A2/DR1 mice were immunized 3 times (two weeks apart) by PMED (gene gun) and the T cell response was measured 7 days after the last immunization by determining interferon-gamma (IFNγ) release by ELISPOT assay. Results for RaDHER2-1 are shown in Tables 2 and 3. Data plot is normalized to the number of cells that secrete IFNγ in 1e6 splenocytes.

Table 2. RaDHER2-1 vaccine induced T cell responses

Peptide pulsed targets and human HER-2+ tumor cells	IFN γ SFC/1e6 splenocytes	
	Human HER2	RaDHER2-1
HBV p18	0 (0)	5(5)
HER-2 p5	38 (9)	358 (23)
HER-2 p106	1148 (22)	770 (11)
HER-2 p98	1176 (56)	806 (10)
K562 A2Kb	5 (3)	4 (3)
K562 A2Kb HER-2+	366 (79)	98 (8)
SKOV3 eGFP	69 (7)	1 (2)
SKOV3 A2+HER-2+	162 (12)	114 (23)

( ) = SD

Table 3. RaDHER2-1 vaccine induced T cell responses

Peptide pulsed target	IFN γ SFC/1e6 splenocytes		
	RaDHER2-1		
	10μg/ml	1μg/ml	0.1μg/ml
HER-2 p106	1156 (18)	1133 (60)	1112 (90)

( ) = SD

As shown in Table 2, RaDHER2-1 vaccine induced T cell responses to 3 human HLA A2 HER2 epitopes p5, p98, p106 as well as tumor cells expressing the endogenously processed human HER2 antigen (K562 A2kb hHER2 and SKOV3 A2+).

The quality of the T cell responses was compared within three different concentrations of p106 epitope in the IFNγ ELISPOT assay, the result being presented in Table 3. As shown in Table 3, the T cell responses induced by RaDHER2-1 vaccine to human HER2 p106 epitope remained high at 0.1 μg/ml of peptide concentration. Together these data confirmed that the RaDHER2-1 antigen can be processed and presented in human MHC class I system, and induce high quality T cell responses to human HER2 antigen.

Results for RaDHER2-2 are shown in Table 4.

Table 4. RaDHER2-2 vaccine induced T cell responses

Peptide pulsed targets and human HER-2+ tumor cells	IFN γ SFC/1e6 splenocytes
HBV p18	0 (0)
HER-2 p5	672 (15)
HER-2 p48	454 (110)
HER-2 p106	2000 (0)
HER-2 p435	2000 (0)
K562 A2Kb	1 (2)
K562 A2Kb-HER2+	1590 (165)

As shown in Table 4, RaD2HER2-2 vaccine induced T cell responses to 3 human HLA A2 HER2 epitopes p5, p48, p106, and p435, as well as tumor cells expressing the endogenously processed human HER2 antigen (K562 A2kb hHER2).

3c. Induction of HER2 specific CD8 T cells in *in vitro* human PBMC system: The ability of RaDHER2-1 DNA when expressed, processed and presented in dendritic cells upon electroporation to induce HER2 specific CD8 T cells was analyzed in a fully human

system using human PBMCs. Briefly, four day differentiated dendritic cells were generated from plastic adherent monocytes isolated from HLA A2 type donor PBMCs in the presence of GMCSF and IL-4. The differentiated dendritic cells were electroporated with RaDHER2-1 plasmid DNA in Nucleofector solution at 5μg/ 100μl containing 1e6 dendritic cells using Amaxa Nucleofector with program U002 and returned to the differentiation medium for another 24hrs at 37°C and 5%CO_2. Autologous CD8 T cells were co- cultured with RaDHER2-1 DNA electroporated dendritic cells at a 5:1 cell ratio supplemented with IL-7 at 10ng/ml and subsequently with IL-10 at 10ng/ml 24hrs later. The CD8 T cells were stimulated again with irradiated and RaDHER2 electroporated dendritic cells as above on day 7 at a CD8:dendritic cell ratio of 10:1. IFNγ ELISPOT was performed seven days after stimulation to confirm the induction of HER2 specific T cells by RaDHER2-1 (data not shown). The epitope specificity of the response was determined after another week of stimulation with adherent autologous monocytes that were irradiated and pulsed with a pool of human HER2 specific peptides (HER2 p48, p106, p369 and p435) and β-2-microglobulin. Approximately 1e5 stimulated CD8 cells from each well were cultured with irradiated T2 cells loaded with corresponding peptide at 10μg/ml for 20hrs at 37°C and 5%CO_2 in IFNγ ELISPOT plates. Plot is normalized to the number of cells that secrete IFNγ from 1e6 CD8 cells. Two independent wells from the same experiment that elicited T cell responses to HER2 peptides are shown. The cultures showed specificity to HER2 p369 (Figure 2a) and p435 peptide sequence (Figure 2b), respectively.

The p435-specific-CD8 culture was further characterized for the ability to recognize HER-2 epitopes presented by cells (K562-A2kb-hHER2) that endogenously express and present HER2 in an HLA A2 restricted manner in an IFNγ ELISPOT assay. Briefly, the p435-specific-CD8 cells were expanded by co-culturing the cells with 2.5e6 partially allogeneic (only HLA A2 matched) and irradiated human PBMCs that have been pulsed with human p435 peptides at a concentration of 10 μg /ml for 2 hours. The cultures were then supplemented with 0.1μg of anti-CD3 antibodies in complete media and incubated at 37°C and 5% CO_2. The cultures were further supplemented with IL-2 at 100IU/ml the next day and at 25IU/ml every 3 days for 14 days prior to the IFNγ ELISOT assay. In the ELISPOT plates, the expanded CD8 cells were incubated with 48 hour IFNγ pretreated K562-A2kb-hHER2 or K562-A2kb cells for 20 hours (Figure 3). Plot is normalized to the number of cells that secrete IFNγ in 1e6 CD8 cells. The p435-specific-CD8 cells elicited IFNγ– response to human HER-2 epitopes presented on the

cells (K562-A2kb-hHER2) when compared to the response to the parental K562-A2kb cells that do not express human HER-2.

Together these data show that the RaDHER2-1 antigen can be processed and presented in human MHC class I system, and induce CD8 cell responses to human HER2 antigen.

CLAIMS

1. An isolated immunogenic HER-2 peptide, or a functional variant thereof, comprising an ECD-derived peptide, wherein the ECD-derived peptide comprises amino acid sequences of at least four of the conserved T cell epitopes in the ECD of human HER-2 protein and shares from 70% to 95% identity with the amino acid sequence of the ECD of human HER-2 protein, and wherein the amino acid sequence of the human HER-2 protein is set forth in SEQ ID NO: 1.

2. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 1, wherein the ECD-derived peptide comprises the amino acid sequences of at least five of the conserved T cell epitopes in the ECD of human HER-2 protein and contains from 50 to 130 amino acid substitutions in regions outside of the conserved T cell epitopes in the ECD of human HER-2 protein, wherein the amino acid substitutions are based on one or more orthologous HER-2 sequences.

3. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 2, wherein the ECD-derived peptide contains between 120 and 130 amino acid substitutions.

4. The isolated immunogenic HER-2 peptide or functional variant thereof of according to claim 2, wherein the ECD-derived peptide contains from 50 to 70 amino acid substitutions.

5. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 1, wherein the ECD-derived peptide comprises amino acids 23 – 645 of SEQ ID NO: 5 or amino acids 25-647 of SEQ ID NO: 14.

6. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 1, wherein the ECD-derived peptide comprises a amino acid sequence that is at least 90% identical to amino acids 23 – 645 of SEQ ID NO: 5 or at least 90% identical to amino acids 25 - 647 of SEQ ID NO: 14.

7. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 1, further comprising a CTD-derived peptide, wherein the C-terminal end of the ECD-derived peptide is joined to the N-terminal end of the CTD-derived peptide.

8. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 7, wherein the CTD-derived peptide comprises amino acids 1023 – 1031 of SEQ ID NO: 1 and shares from 70% to 95% identity with the amino acid sequence of the CTD domain of the human HER-2 protein.

9. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 8, wherein the CTD-derived peptide comprises from 15 to 70 amino acid substitutions from the CTD domain of one or more orthologous HER-2 proteins.

10. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 7, wherein the CTD-derived peptide comprises the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO:22.

11. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 10, wherein the ECD-derived peptide comprises amino acids 23 – 645 of SEQ ID NO: 5 or amino acids 25-647 of SEQ ID NO.: 14.

12. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 1, comprising amino acids 23 – 910 of SEQ ID NO. 3 or amino acids 25-912 of SEQ ID NO: 18.

13. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 7, further comprising a TMD-derived peptide, wherein the carboxy terminus of the ECD-derived peptide is joined to the amino terminus of the TMD-derived peptide and the carboxy terminus of the TMD-derived peptide is joined to the amino terminus of the CTD-derived peptide.

14. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 13, wherein the TMD-derived peptide comprises the amino acid sequence of SEQ ID NO: 11 or the amino acid sequence of SEQ ID NO: 16.

15. The isolated immunogenic HER-2 peptide or functional variant thereof according to claim 14, which comprises amino acids 23 - 940 of SEQ ID NO: 9 or amino acids 25 – 956 of SEQ ID NO: 20.

16. A composition comprising an isolated immunogenic HER-2 peptide or a functional variant thereof according to any of claims 1 – 15, and a pharmaceutically acceptable excipient.

17. An isolated nucleic acid molecule comprising a polynucleotide that encodes an isolated immunogenic HER-2 peptide according to any of claims 1 – 15.

18. The isolated nucleic acid molecule according to claim 17, wherein the nucleotide sequence encodes an amino acid sequence selected from the group consisting of:

(a) amino acids 23 - 910 of SEQ ID NO: 3;

(b) amino acids 23 - 645 of SEQ ID NO: 5;

(c) amino acids 23 - 940 of SEQ ID NO: 9;

(d) amino acids 1- 623 of SEQ ID NO: 12;

(e) amino acids 25 - 647 of SEQ ID NO: 14;

(f) amino acids 25 - 912 of SEQ ID NO: 18; and

(g) amino acids 25 – 956 of SEQ ID NO: 20.

19. The isolated nucleic acid molecule according to claim 17, wherein the nucleotide sequence is selected from the group consisting of nucleotide sequence of SEQ IDS NOs: 4, 6, 10, 13, 15, 19, and 21.

20. A plasmid construct comprising an isolated nucleic acid molecule according to claim 17.

21. A composition comprising a nucleic acid molecule according to claim 17.

22. A method of inhibiting abnormal cell proliferation in a mammal, comprising administering to the mammal an effective amount of a composition according to claim 16.

23. A method of inhibiting abnormal cell proliferation in a mammal comprising administering to the mammal an effective amount of a composition according to claim 21.

24. A method of treating or preventing cancer in a mammal, comprising administering to the mammal an effective amount of a composition according to claim 16.

25. A method of treating or preventing cancer in a mammal comprising administering to the mammal an effective amount of a composition according to claim 21.

26. The method according to claim 24 or 25, wherein the cancer is a cancer associated with HER-2.

27. The method according to claim 26, wherein the cancer is breast cancer or ovary cancer.

28. The method according to claim 26, wherein the composition is administered in combination with one or more other therapeutic agents capable of inhibiting abnormal cell proliferation.

29. A method of eliciting an immune response in a mammal, comprising administering to the mammal an effective amount of a composition according to claim 16.

30. A method of eliciting an immune response in a mammal, comprising administering to the mammal an effective amount of a composition according to claim 21.

FIG. 1

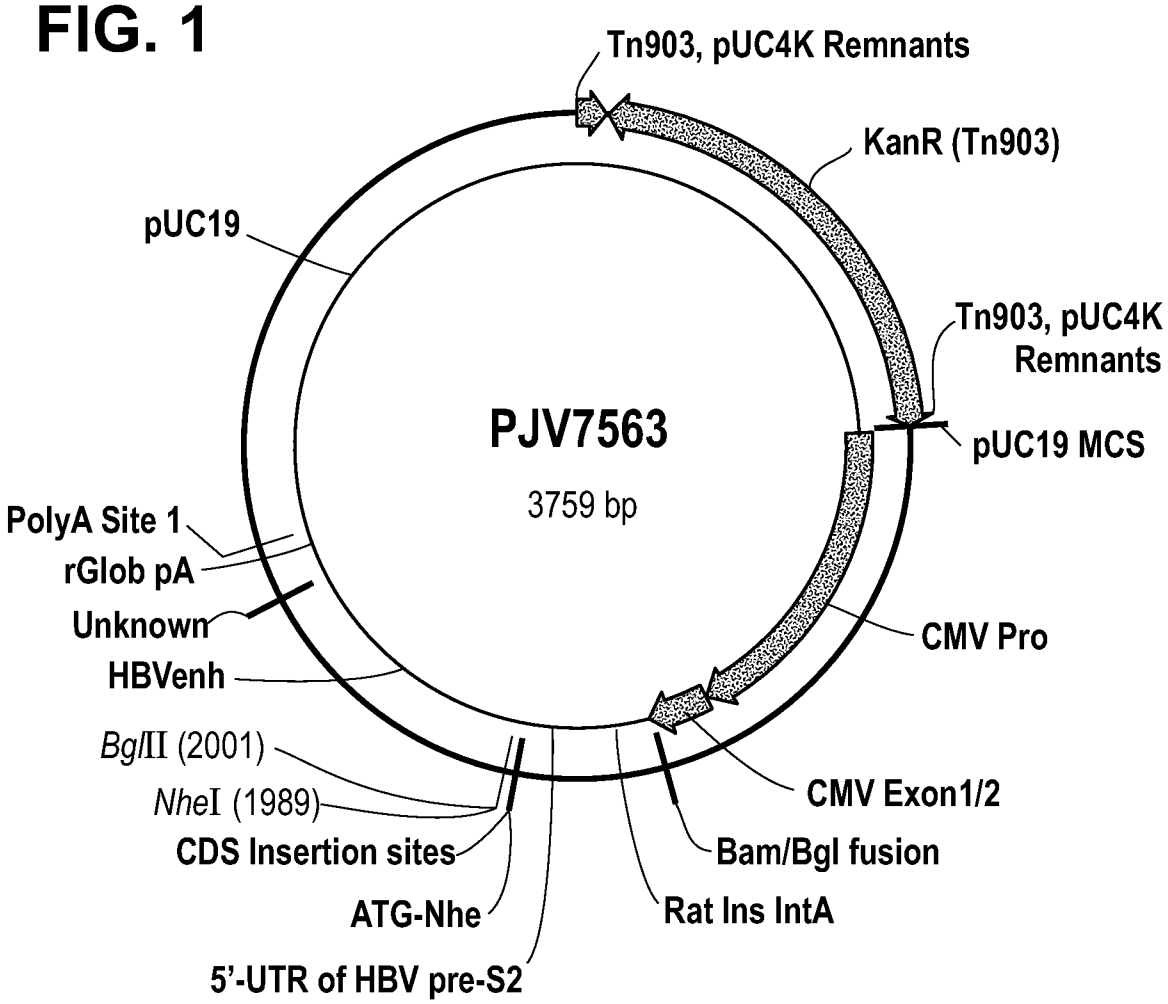

Tn903, pUC4K Remnants
KanR (Tn903)
pUC19
Tn903, pUC4K Remnants
pUC19 MCS
PJV7563
3759 bp
PolyA Site 1
rGlob pA
Unknown
HBVenh
CMV Pro
BglII (2001)
NheI (1989)
CDS Insertion sites
CMV Exon1/2
Bam/Bgl fusion
ATG-Nhe
Rat Ins IntA
5'-UTR of HBV pre-S2

FIG. 2A

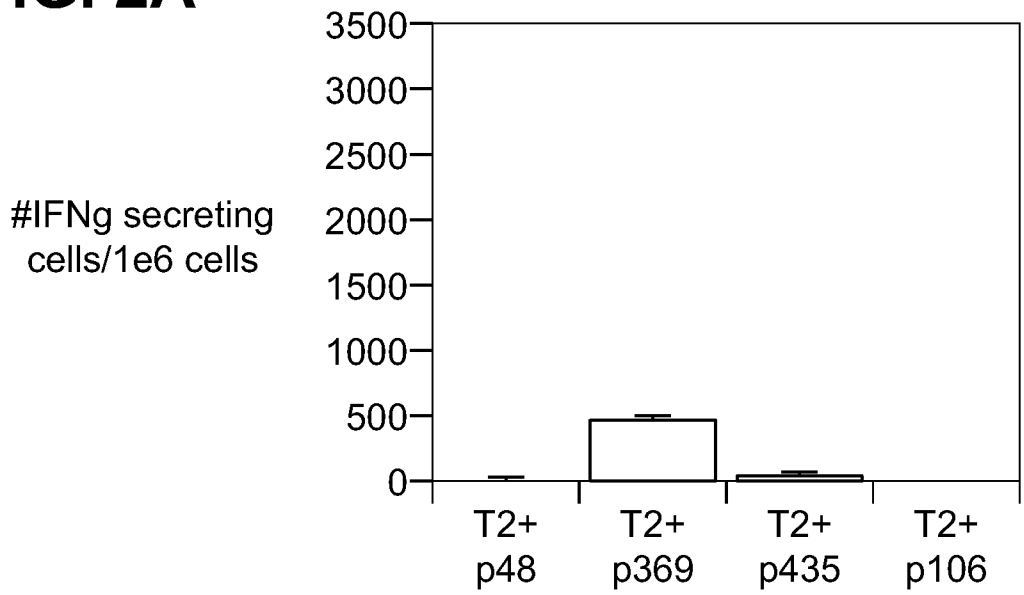

#IFNg secreting cells/1e6 cells
3500
3000
2500
2000
1500
1000
500
0
T2+ p48
T2+ p369
T2+ p435
T2+ p106

FIG. 2B

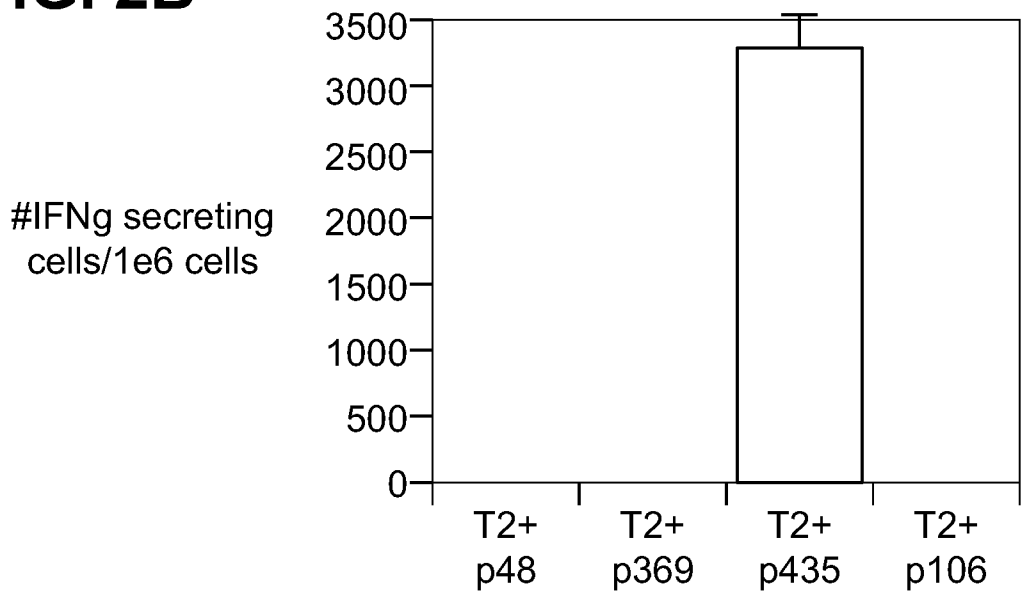
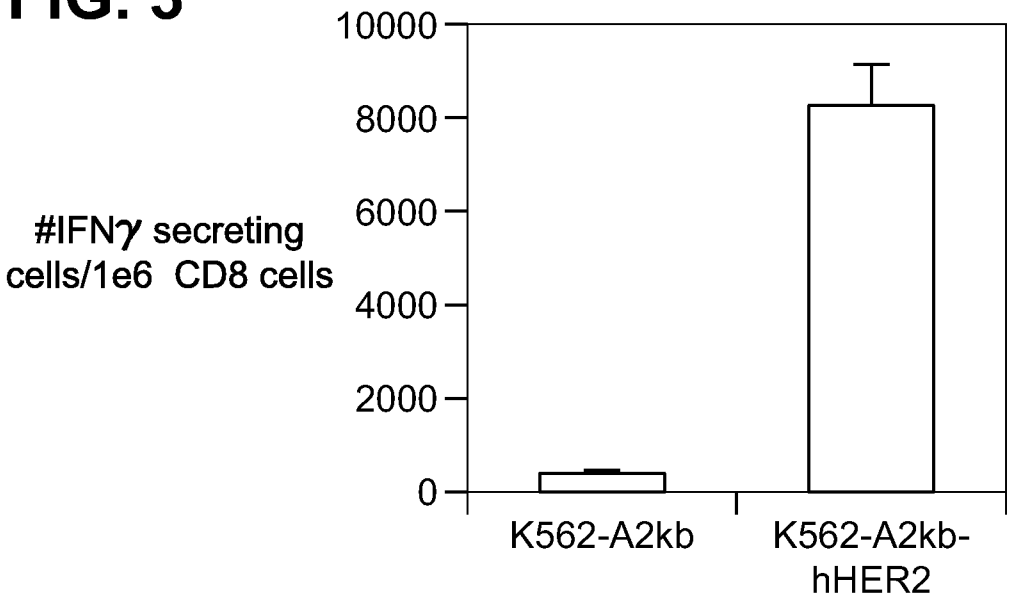

#IFNg secreting cells/1e6 cells
3500
3000
2500
2000
1500
1000
500
0
T2+ p48
T2+ p369
T2+ p435
T2+ p106

FIG. 3


#IFNγ secreting cells/1e6 CD8 cells
10000
8000
6000
4000
2000
0
K562-A2kb
K562-A2kb-hHER2

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2011/052246

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/71 C07K14/82 A61K39/00 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HSU W L ET AL: "Increased survival in dogs with malignant mammary tumours overexpressing HER-2 protein and detection of a silent single nucleotide polymorphism in the canine HER-2 gene", VETERINARY JOURNAL, BAILLIERE TINDALL, LONDON, GB, vol. 180, no. 1, 1 April 2009 (2009-04-01) , pages 116-123, XP025964978, ISSN: 1090-0233, DOI: 10.1016/J.TVJL.2007.10.013 [retrieved on 2007-12-03] the whole document ----- -/--	1-30

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

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2011	18/10/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Valcárcel, Rafael

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2011/052246

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CONSTANTIN N BAXEVANIS ET AL: "Immunogenic HER-2/neu peptides as tumor vaccines", CANCER IMMUNOLOGY, IMMUNOTHERAPY, SPRINGER, BERLIN, DE, vol. 55, no. 1, 27 October 2005 (2005-10-27), pages 85-95, XP019422474, ISSN: 1432-0851, DOI: 10.1007/S00262-005-0692-3 the whole document	1-30
X	DISIS M L ET AL: "GENERATION OF IMMUNITY TO THE HER-2/NEU ONCOGENIC PROTEIN IN PATIENTS WITH BREAST AND OVARIAN CANCER USING A PEPTIDE-BASED VACCINE", CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 5, no. 6, 1 January 1999 (1999-01-01) , pages 1289-1297, XP000914565, ISSN: 1078-0432 the whole document	1-30
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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