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(72) Feltaláló(k):
FISCHER, Laurent, Bernard, 69110 Sainte Foy Les Lyon (FR)

(73) Jogosult(ak):
Merial, Inc., Duluth, GA 30096 (US)

(74) Képviselő:
Danubia Szabadalmi és Jogi Iroda Kft., Budapest

(54) **HER2 DNS vakcina kedvtelésből tartott állatok rákjának kiegészítő kezeléseként**

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(54) **HER2 DNA VACCINE AS ADJUNCT TREATMENT FOR CANCERS IN COMPANION ANIMALS**

HER2 DNA IMPSTOFF ALS ZUSATZTHERAPIE VON KREBSERKRANKUNGEN IN HEIMTIERE

VACCIN D'ADN HER2 COMME TRAITEMENT AUXILIAIRE DES CANCERS CHEZ LES ANIMAUX DE COMPAGNIE

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(73) Proprietor: **Merial, Inc.**
Duluth, GA 30096 (US)

(72) Inventor: **FISCHER, Laurent, Bernard**
69110 Sainte Foy Les Lyon (FR)

(74) Representative: **D Young & Co LLP**
120 Holborn
London EC1N 2DY (GB)

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Description

BACKGROUND OF THE INVENTION

[0001] This application relates to compositions for treatment of differentiation antigen-dependent cancers and to methods of using such compositions. The invention utilizes compositions containing xenogeneic differentiation antigens, which are associated with cancers to provide effective therapy.

[0002] Differentiation antigens are tissue-specific antigens that are shared by autologous and some allogeneic tumors of similar derivation, and on normal tissue counterparts at the same stage of differentiation. Differentiation antigens have been shown to be expressed by a variety of tumor types, including melanoma, leukemia, lymphomas, colorectal carcinoma, breast carcinoma, prostate carcinoma, ovarian carcinoma, pancreas carcinomas, and lung cancers. For example, differentiation antigens expressed by melanoma cells include Melan-A/MART-1, Pme117, tyrosinase, and gp75. Differentiation antigen expressed by lymphomas and leukemia include CD 19 and CD20/CD20 B lymphocyte differentiation markers). An example of a differentiation antigen expressed by colorectal carcinoma, breast carcinoma, pancreas carcinoma, prostate carcinoma, ovarian carcinoma, and lung carcinoma is the mucin polypeptide muc-1. A differentiation antigen expressed by, for example, breast carcinoma is Her2 (synonyms: Her2/neu, ECBB2, ErbB2, c-erb-2), which is a gene coding for a tyrosine kinase receptor that is a member of the family of epidermal growth factor receptors (De Maria et al., 2005). Over expression of Her2 has been demonstrated in mammary gland tumors of both the cat (Winston et al., 2005) and the dog (Rungsipipat et al., 2008). Winston et al. (2005) used existing assay methods (HERCEPTEST™, Dako USA, Carpinteria, CA; NCL-CB11, Novocastra, Newcastle, UK) to successfully grade levels of Her2 expression on feline mammary tumors as 0=minimal/absent, 1=weak, 2=moderate, or 3=intense. The HERCEPTEST™ and NCL-CB11 assays identified 27 and 23 cats respectively, out of 30 examined, as having grade 2 or 3 Her2 expression in mammary tumor samples.

[0003] In addition to successfully grading levels of Her2 over expression in feline mammary tumors, Winston et al. (2005) used the HERCEPTEST ™ to detect low levels of Her2 expression in normal feline epithelial tissues and cell types including: hair follicle, mammary gland, gastric pit, salivary gland duct, renal cortical and medullary tubules, colonic and small intestinal crypt, brain, pancreatic duct and islets, splenic macrophages, adrenal cortex, hepatocytes, and testicular Leydig's cells. Expression of Her2 has been documented on a range of human epithelial cell types including gastro-intestinal, respiratory, reproductive, urinary, skin, mammary and placenta (Press et al., 1990). These findings indicate that the expression of Her2 is common in a range of tissue types of humans and cats. The finding of Her2 over expression in dog mammary tumors suggests this species would share expression characteristics identified in humans and cats. Existing assays and reagents can serve as tools to screen expression levels of Her2 in companion animal cancers in order to justify treatment with the Her2 cancer vaccine.

[0004] Unfortunately, in most cases, the immune system of the individual is tolerant of such differentiation antigens, and fails to mount an effective immune response. Several technologies have been considered to address this challenge: (cytokines as genetic adjuvants (Chang et al., 2004), xenogeneic vaccination (Pupa et al., 2005), electrotransfer (Quaglino et al., 2004), combination with chemotherapy (Bernhardt et al., 2002). Although results were encouraging, greater efficacy was required for these approaches to be considered a key component of a first-line therapeutic strategy. Further, recent findings indicate both antibody and cell-mediated immunity are required for tumor eradication post immunization, perhaps explaining, in part, the lack of success in the field (Orlandi et al., 2007). Therefore, for the treatment of cancers where the tumor expresses differentiation antigens therefore, it would be desirable to have a method for stimulating a therapeutically effective immune response against the differentiation antigen in vivo. It an object of the present invention to provide such a method.

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D. Peruzzi et al, Vaccine 28 (2010), pp 1201-1208 describes telomerase and HER-2/neu as targets of genetic cancer vaccines in dogs.

SUMMARY OF THE INVENTION

[0006] It has now been found that the tolerance of the immune system for self-derived target differentiation antigens can be overcome and an immune response stimulated by administration of a xenogeneic differentiation antigen (wild-type or mutant) of the same type from a species different from the subject being treated (US 6,328,969 & US 7,556,805 , to Sloan-Kettering).

[0007] For example, a rat differentiation antigen can be used to stimulate an immune response to the corresponding differentiation antigen in a canine subject. Administration of altered antigens in accordance with the invention results in an effective immunity against the original antigen expressed by the cancer in the treated subject Thus, in accordance with a first aspect of the invention, there is provided a xenogeneic Her2/neu antigen that is xenogeneic to a Her2/neu antigen expressed by mammary cells of a dog for use in the treatment of canine mammary carcinoma/tumor in a dog suffering from canine mammary carcinoma/tumor, wherein the xenogeneic Her2/neu antigen is in an immunological-effective amount and the xenogeneic Her2/neu antigen is administered post-surgical removal of tumors in subjects suffering from said cancers, and wherein the xenogenic mammary gland tumor-associated Her2/neu antigen is a vector comprising a DNA sequence encoding the xenogeneic therapeutic mammary gland tumor-associated Her2/neu antigen under the control of a promoter which promotes expression of the mammary gland tumor-associated Her2/neu antigen in the dog. Also provided is a xenogeneic Her2/neu antigen that is xenogeneic to a Her2/neu antigen expressed by mammary gland cells of a dog for use in the treatment of canine mammary gland tumor in a dog suffering from canine mammary gland carcinoma/tumor, wherein the xenogeneic mammary gland carcinoma/tumor-associated Her2/neu antigen is a vector comprising a DNA sequence encoding the xenogeneic therapeutic mammary gland carcinoma/tumor-associated Her2/neu antigen under the control of a promoter which promotes expression of the xenogeneic mammary gland tumor-associated Her2/neu antigen in the dog, and wherein the vector has the sequence comprising 106-3885 of the sequence as set forth in SEQ IDNO:1 and the xenogeneic Her2/neu antigen is administered post-surgical removal of tumors in subjects suffering from said cancers.

Further provided is a vector that is capable of expressing in vivo in a canine the protein as set forth in SEQ ID NO:2 for use in the treatment of canine mammary carcinoma/tumors in a dog suffering from canine mammary carcinoma/tumor, wherein the canine mammary carcinoma/tumor is a Her2/neu associated mammary carcinoma/tumor, wherein the vector is administered post-surgical removal of tumors in subjects suffering from said cancers.

Further provided is use of a xenogeneic Her2/neu antigen that is xenogeneic to a Her2/neu antigen expressed by mammary cells of a dog for the manufacture of a medicament for the treatment of canine mammary carcinoma/tumor in a dog suffering from canine mammary carcinoma/tumor, wherein the xenogeneic Her2/neu antigen is in an immunological effective amount and the xenogeneic Her2/neu antigen is administered post-surgical removal of tumors in subjects suffering from said cancers, and wherein the xenogenic mammary gland tumor-associated Her2/neu antigen is a vector comprising a DNA sequence encoding the xenogeneic therapeutic mammary gland tumor-associated Her2/neu antigen under the control of a promoter which promotes expression of the mammary gland tumor-associated Her2/neu antigen in the dog.

As an additional aspect provided is use of a xenogeneic Her2/neu antigen that is xenogeneic to a Her2/neu antigen expressed by mammary gland cells of a dog for the manufacture of a medicament for the treatment of canine mammary gland tumor in a dog suffering from canine mammary gland carcinoma/tumor, wherein the xenogeneic mammary gland carcinoma/tumor-associated Her2/neu antigen is a vector comprising a DNA sequence encoding the xenogeneic therapeutic mammary gland carcinoma/tumor-associated Her2/neu antigen under the control of a promoter which promotes expression of the xenogeneic mammary gland tumor-associated Her2/neu antigen in the dog, and wherein the vector has the sequence comprising 106-3885 of the sequence as set forth in SEQ ID NO:1 and the xenogeneic Her2/neu antigen is administered post-surgical removal of tumors in subjects suffering from said cancers.

The scope of the present invention is defined by the claims. Subject Matter outside the scope of the claims is for information only.

[0008] Therapeutic differentiation antigens based on mammary gland carcinoma/tumor-associated differentiation an-

tigens are used in accordance with the invention to treat, for example, mammary gland carcinoma post-surgical removal of tumors in subjects suffering from said cancers. In one embodiment of the invention, a plasmid comprising a sequence encoding a xenogeneic tyrosine kinase receptor, for example rat tyrosine kinase receptor, under the control of a suitable promoter, is administered to a subject. For example, dogs have been treated using plasmids comprising a DNA sequence encoding rat tyrosine kinase receptor with pronounced clinical benefit.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009]

FIG. 1A shows overall survival time post-immunization and surgical resection of MGT;
FIG. 1B shows disease-free survival time post-immunization and surgical resection of MGT;
FIG. 1C shows metastasis-free survival time post-immunization and surgical resection of MGT;
FIG. 2 shows a map of the pcDNA3.1 (+/-) plasmid
FIG. 3 shows a map and sequence for the pINGhumanTyrosinase plasmid, where the coding sequence for the human tyrosinase has been removed This is where the rat Her2/neu (nucleotides 17-3799 of SEQ ID NO: 1) was inserted to produce rHer2/neu-pING of the instant invention.

DETAILED DESCRIPTION OF THE INVENTION

[0010] The present invention provides a method for treating mammary gland tumors in a subject by stimulating an immune response to a mammary gland-associated differentiation antigen. The subject is preferably canine or feline, although the invention can be applied to other animal species, preferably mammalian or avian species, as well.

[0011] As used in the specification and claims of this application, the term "immune response" encompasses both cellular and humoral immune responses. Preferably, the immune response is sufficient to provide immunoprotection against growth of tumors expressing the target differentiation antigen. The term "stimulate" refers to the initial stimulation of a new immune response or to the enhancement of a pre-existing immune response.

[0012] In accordance with the invention, a subject is treated by administering a xenogeneic differentiation antigen of the same type as a target differentiation antigen expressed by mammary gland tumor cells of the subject in an amount effective to stimulate an immune response. Thus, the target differentiation antigen is the Her2/neu antigen found in mammary cells, and the therapeutic antigen is a xenogeneic Her2/neu antigen.

[0013] In one embodiment, the inventive use may include the following steps: (1) immunization to an animal in need of a xenogeneic antigen, for example, the rat Her2/neu as set forth in SEQ ID NO:2 and encoded by nucleotides 106-3885 of the sequence as set forth in SEQ ID NO:1, (2) needle-free priming of immune responses, (3) electrotransfer-based booster, and (4) vaccination after tumor debulking by surgical primary therapy.

[0014] In another embodiment, the inventive use is carried out on subjects, including companion animals, without metastasis (i.e. in relatively early stages of mammary carcinoma disease progression).

[0015] In some embodiments, the boost comprises administering plasmids encoding xenogeneic antigens, for example those encoding rat Her2 protein (SEQ ID NO:2).

[0016] In some embodiments, the xenogeneic antigen is encoded by a nucleotide having favorable nucleotide substitutions with respect to the sequence as set forth in SEQ ID NO:1. Favorable substitutions include any changes that result in improved immune response against the Her2/neu expressed by the cells of the mammary tumor/carcinoma. Substitutions can include existing sequences, such as murine Her2 (SEQ ID NO:3), human Her2 (SEQ ID NO:4), or any other xenogeneic Her2 sequence, or fragment thereof, capable of eliciting a therapeutically effective immune response in a target animal against a Her2-associated mammary carcinoma.

[0017] In some embodiments, the boost comprises administering a xenogeneic differentiation antigen.

[0018] In other embodiments, the boost comprises administering a syngeneic differentiation antigen.

[0019] Xenogeneic differentiation antigen may be administered as a purified differentiation antigen derived from the source organism. Proteins can be purified for this purpose from cell lysates using column chromatography procedures. Proteins for this purpose may also be purified from recombinant sources, such as bacterial or yeast clones or mammalian or insect cell lines expressing the desired product.

[0020] Administration of the xenogeneic differentiation antigen can be accomplished by several routes. First, the xenogeneic differentiation antigen may be administered as part of a vaccine composition which may include one or more adjuvants such as alum, QS21, TITERMAX or its derivatives, incomplete or complete Freund's and related adjuvants, and cytokines such as granulocyte-macrophage colony stimulating factor, flt-3 ligand, interleukin-2, interleukin-4 and interleukin-12 for increasing the intensity of the immune response. The vaccine composition may be in the form of a xenogeneic differentiation antigen in a solution or a suspension, or the therapeutic differentiation antigen may be introduced in a lipid carrier such as a liposome. Such compositions will generally be administered by subcutaneous, intradermal

or intramuscular route. Vaccine compositions containing expressed xenogeneic differentiation antigen are administered in amounts which are effective to stimulate an immune response to the target differentiation antigen in the subject. The preferred amount to be administered will depend on the species of the subject and on the specific antigen, but can be determined through routine preliminary tests in which increasing doses are given and the extent of antibody formation or T cell response is measured by ELISA or similar tests. T cell responses may also be measured by cellular immune assays, such as cytotoxicity, cytokine release assays and proliferation assays.

[0021] The xenogeneic differentiation antigen may also be introduced in accordance with the invention using a DNA immunization technique in which DNA encoding the antigen is introduced into the subject such that the xenogeneic differentiation antigen is expressed by the subject. cDNA encoding the differentiation antigen is combined with a promoter which is effective for expression of the nucleic acid polymer in mammalian cells. This can be accomplished by digesting the nucleic acid polymer with a restriction endonuclease and cloning into a plasmid containing a promoter such as the SV40 promoter, the cytomegalovirus (CMV) promoter or the Rous sarcoma virus (RSV) promoter. The resulting construct is then used as a vaccine for genetic immunization. The nucleic acid polymer could also be cloned into plasmid and viral vectors that are known to transduce mammalian cells. These vectors include retroviral vectors, adenovirus vectors, vaccinia virus vectors, pox virus vectors and adenovirus-associated vectors.

[0022] The nucleic acid constructs containing the promoter and the antigen-coding region can be administered directly or they can be packaged in liposomes or coated onto colloidal gold particles prior to administration. Techniques for packaging DNA vaccines into liposomes are known in the art, for example from Murray, ed. "Gene Transfer and Expression Protocols" Humana Pres, Clifton, N.J. (1991). Similarly, techniques for coating naked DNA onto gold particles are taught in Yang, "Gene transfer into mammalian somatic cells in vivo", Crit. Rev. Biotech. 12: 335-356 (1992), and techniques for expression of proteins using viral vectors are found in Adolph, K. ed. "Viral Genome Methods" CRC Press, Florida (1996).

[0023] For genetic immunization, the vaccine compositions are preferably administered intradermally, subcutaneously or intramuscularly by injection or by gas driven particle bombardment, and are delivered in an amount effective to stimulate an immune response in the host organism. The compositions may also be administered ex vivo to blood or bone marrow-derived cells (which include APCs) using liposomal transfection, particle bombardment or viral infection (including co-cultivation techniques). The treated cells are then reintroduced back into the subject to be immunized. While it will be understood that the amount of material needed will depend on the immunogenicity of each individual construct and cannot be predicted *a priori,* the process of determining the appropriate dosage for any given construct is straightforward. Specifically, a series of dosages of increasing size, starting at about 0.1 μg is administered and the resulting immune response is observed, for example by measuring antibody titer using an ELISA assay, detecting CTL response using a chromium release assay or detecting TH (helper T cell) response using a cytokine release assay.

[0024] Once tolerance is broken through the administration of the xenogeneic differentiation antigen, subsequent treatments with syngeneic differentiation may be employed to maintain and in some cases enhance the immune response. (See, Weber, et al., "Tumor immunity and autoimmunity induced by immunization with homologous DNA." J Clin Invest 102 (6):1258 (1998).) Thus, in one embodiment of the invention, the subject is first treated by administration of a xenogeneic differentiation antigen (for example for three treatment cycles), and subsequently by administration of a syngeneic differentiation antigen (for example for an additional three treatment cycles). As an alternative to treatment cycles using different therapeutic agents, one can use a single therapeutic agent containing both xenogeneic and syngeneic differentiation antigens. Thus, for example, a mixture of the rHer2-pING and hHer2-pING vectors, or a single vector encoding both rat and human Her2/neu under the control of a promoter such that they are expressed in a canine subject can be employed for the treatment of mammary gland tumor in canines. Vectors are available commercially, for example from Stratagene and other companies, which can express two independent genes. Commonly, these vectors use an internal ribosomal entry site, or IRES, between the two genes. This approach has the advantage of requiring approval for only a single therapeutic agent.

[0025] The invention will now be further described with reference to the following, non-limiting examples.

Example 1- Her2/neu expression plasmid construction

[0026] The extracellular domain of rat HER2/neu (nucleotides 17-3799 of SEQ ID NO:1) was amplified by PCR from the pCMVneuNT (Amici et al., 1998) plasmid using the primers forward: 5'-CGAAGCTTACCATGGAGCTGGCGGCCTGG-3' (SEQ ID NO:6) and reverse: 5'-CGGAATTCTTATGTCACCGGGCTGGC-3' (SEQ ID NO:7). The HindIII-EcoRI fragment was cloned into pcDNA3.1(+) (Invitrogen, Carlsbad, CA; and FIG. 2). The original sequence of the rat neu cDNA was described previously (Bargmann et al., 1986), and is herein set forth in SEQ ID NO:1, with the coding sequence from nucleotides 17 to 3799. The rat HER2/neu coding sequence was then subcloned into the pING vector (Bergman et al., Clin Cancer Res, 9: 1284-1290, 2003, backbone depicted in FIG. 3; map depicted in FIG. 3A; and sequence as set forth in SEQ ID NO:5), to yield rat HER2/neu-pING.

Example 2 - Immunization of Mammary Gland Tumor (MGT)-positive canines with pING-rHer2

[0027] In this trial, 10 dogs with MGT were enrolled and immunized with 100 μg of pING-rHer2 DNA per dose. The signalment for these dogs is set forth in Table 1 and the tumor staging is set forth in Table 2.

Table 1. Trial animal characteristics

	Age (yrs)	Breed	Weight (kg)
MGT 01	9	Yorkshire Terrier	1.75
MGT 02	13	Mixed	9.8
MGT 03	12	Yorkshire Terrier	5
MGT 04	7	Lhasa Apso	11
MGT 05	10	Maltese	3.35
MGT 06	12	Cavalier King Charles Spaniel	9
MGT 07	8	Pomeranian	2.8
MGT08	12	Maltese	3.9
MGT09	13	Pomeranian	2.7
MGT10	12	Yorkshire Terrier	3
Median	**12**	-	**3.6**

Table 2. Tumor staging

	Tumor size (cm)	MGT Type	Stage
MGT 01	2x2x4 0.2x0.2x0.2 0.2x0.3x0.2 0.1x0.1x0.1 0.5x0.5x0.5 0.2x0.2x0.2 0.5x0.5x0.5	Tubulopapillary carcinoma	$T_3N_0M_0$
MGT 02	12x10x8 5x3x1.5 1x1x1 1x1x0.5 0.5x0.1x0.1	Lipid rich carcinoma	$T_3 N_0M_0$
MGT 03	5.6x4.8x4.6 1.8x1.5x1.2	Tubulopapillary carcinoma with fibroadenoma	$T_3N_0M_0$
MGT 04	4.2x5.6x2.5	Tubulopapillary carcinoma	$T_3 N_0M_0$
MGT 05	1.2x1x0.5 1x 1.4x0.5 1x1x0.4 0.5x0.5x0.5	Simple adenoma	$T_1N_0M_0$
MGT 06	10x4x3	Lipid rich carcinoma with fibroadenoma	$T_3N_0M_0$
MGT 07	1x1x1 0.5x0.5x0.5	Complex type	$T_1N_0M_0$
MGT08	1x1x1 0.5x0.5x0.5	Complex type	$T_1N_0M_0$
MGT09	2.5x2x1 1.5x2x1	Complex type	$T_1N_0M_0$
MGT10	1x1x1 0.5x0.5x0.5 0.1x0.1x0.1	Tubulopapillary carcinoma	$T_1N_0M_0$

[0028] As indicated, this group included five stage I and five stage III dogs, which all received three doses of vaccine at two week intervals. The first and second doses were administered with the VITAJET™ transdermal device and the third dose by intramuscular injection concurrent with electroporation. Vaccination was initiated following surgical removal of the MGT with concurrent ovariohysterectomy (OHE). All dogs were negative for regional lymph node and pulmonary metastasis. Disease free survival and overall survival times were calculated using day of surgery as day 0 with results presented in Table 3. Table 3. Disease-free and overall survival time

Dog	WHO Stage	Disease-free survival		Overall survival time (days)	Outcome
		recurrence	metastasis		
MGT 05	I	703	703	703	alive
MGT 07	I	669	669	669	alive
MGT 08	I	548	548	548	alive
MGT 09	I	536	536	536	alive
MGT 10	I	482	482	482	dead
Stage I Dogs		**548**	**548**	**548**	---
MGT 01	III	779	779	779	alive
MGT 02	III	212	182	212	dead
MGT 03	III	762	762	762	alive
MGT 04	III	575	381	720	alive w/ met
MGT 06	III	686	686	686	alive
Stage III Dogs		**686**	**686**	**720**	---
All Dogs Median		**622**	**609**	**678**	

[0029] A group of 19 dogs was identified as historical control cases. All control dogs underwent surgical removal of MGT with concurrent OHE and were negative for regional lymph node and pulmonary metastasis. This group included 7 stage I, 3 stage II, and 9 stage III dogs. Disease free and overall survival times were calculated for these dogs using day of surgery as day 0. The signalment for these dogs is set forth in Table 4 and tumor staging for each dog is set forth in Table 5. Disease free and overall survival times were calculated for the control group and are presented in FIGs 1A-1C.

Table 4. Control dog signalment

	Case Number	Age (yrs)	Breed	Weight (kg)
1	9403460	7	Mix	1.75
2	9404023	14	Poodle	2.5
3	9405132	14	Yorkshire	2.3
4	9409179	12	Finnish Spitz	6.8
5	9409043	14	Poodle	3.2
6	9500057	9	Lhasa Apso	6.5
7	9500890	14	Maltese	6
8	9500959	15	Cocker	14
9	923543	11	Siberian Huskies	16
10	9405082	13	Poodle	3.9
11	9505202	9	Mix	12
12	9600998	10	Maltese	4.6
13	9700451	13	Maltese	2.7
14	892285	12	Yorkshire	1.6
15	9502927	14	Maltese	3.2
16	9405356	10	Cocker	12
17	9409104	11	Maltese	3.8
18	9503957	6	Miniature Schnauzer	4

(continued)

	Case Number	Age (yrs)	Breed	Weight (kg)
19	9404023	14	Poodle	3
	Median	**12**		3.9

Table 5. Tumor staging for control dogs

	Clinical NO.	Tumor size	MGT Type	Stage
1	9403460	6x6x7	Complex carcinoma	$T_3 N_0M_0$
2	9404023	3x3x3	Squamous cell carcinoma	$T_2N_0M_0$
3	9405132	7x4x7 2x2x2 0.3x0.2x0.2 0.5x0.5x0.5	Simple or complex carcinoma	$T_3N_0M_0$
4	9409179	13x12x12 6x7x7 1x1x1	Simple carcinoma with squamous cell carcinoma	$T_3N_0M_0$
5	9409043	3.5x2.x1 3x1.5x1	Tubulopapillary carcinoma	$T_2N_0M_0$
6	9500057	3x2x2 2x1x1	Tubulopapillary carcinoma	$T_2N_0M_0$
7	9500890	8x3x1	Simple carcinoma	$T_3N_0M_0$
8	9500959	8x3x2 2x1x0.5	Adenocarcinoma	$T_3N_0M_0$
9	923543	5x5x4 0.2x0.2x0.2	Simple carcinoma	$T_3N_0M_0$
10	9405082	5x4x3.5 3x3.5x3	Simple carcinoma	$T_3N_0M_0$
11	9505202	0.3x0.3x0.3 1x1x0.5 0.4x0.4x0.4	Tubulopapillary carcinoma	$T_1N_0M_0$
12	9600998	0.5x0.5x0.4 1x0.5x0.5	Carcinoma	$T_1N_0M_0$
13	9700451	1x1x1 1x1x1	Tubulopapillary carcinoma	$T_1N_0M_0$
14	892285	0.5x0.8x0.3 1x0.8x0.5	Carcinoma in benign mixed tumor	$T_1N_0M_0$
15	9502927	5x4x4 0.5x0.5x0.5	Carcinoma in benign mixed tumor	$T_3N_0M_0$
16	9405356	10x3x1.5	Tubulopapillary carcinoma	$T_3N_0M_0$
17	9409104	1x1x1 0.5x0.5x0.5 2x2x2	Adenocarcinoma	$T_1N_0M_0$
18	9503957	2x2x2 0.3x0.3x0.3	Adenocarcinoma, complex type	$T_1N_0M_0$
19	9404023	2x2x1	Adenocarcinoma,	$T_1N_0M_0$

[0030] Philibert et al. (2003) reviewed survival statistics for 97 dogs with MGT and reported median survival times for 41 dogs with MGT less than 3 cm in diameter to be 22 months (~666 days) versus 14 months (~424 days) for 56 dogs with MGT greater than 3 cm in diameter. In the absence of lymph node involvement or metastasis, tumor size less than 3 cm correlates with stage I disease and greater than 3 cm correlates with stage II or higher disease status. They did not find a difference in survival time for dogs in stages II, III or IV.

[0031] Overall median survival time for all dogs treated with the pING-rHer2 vaccine is 678 days. This was significantly higher as compared to the historical data from the 19 dogs provided by NTU indicating a median overall survival time of 300 days, and to the data published by Philibert et al. (2003) indicating 424 days overall survival time for dogs with stage II or greater MGT.

[0032] The pING-rHer2 DNA vaccine will target dogs and cats with tumors shown to over express the Her2 antigen based upon tumor tissue analysis using existing Her2 tissue expression assays. The vaccine will be administered using the Vetjet™ transdermal device to deliver 100 μg of DNA into the medial thigh of dogs or lateral thigh of cats, at two week intervals for four doses. Dogs and cats that survive will receive a booster dose every six months.

Claims

1. A xenogeneic Her2/neu antigen that is xenogeneic to a Her2/neu antigen expressed by mammary cells of a dog for use in the treatment of canine mammary carcinoma/tumor in a dog suffering from canine mammary carcinoma/tumor, wherein the xenogeneic Her2/neu antigen is in an immunological-effective amount and the xenogeneic Her2/neu antigen is administered post-surgical removal of tumors in subjects suffering from said cancers, and wherein the xenogenic mammary gland tumor-associated Her2/neu antigen is a vector comprising a DNA sequence encoding the xenogeneic therapeutic mammary gland tumor-associated Her2/neu antigen under the control of a promoter which promotes expression of the mammary gland tumor-associated Her2/neu antigen in the dog.

2. The xenogeneic Her2/neu antigen according to claim 1 for use according to claim 1, wherein the xenogeneic mammary gland tumor-associated differentiation antigen is rat Her2/neu.

3. A xenogeneic Her2/neu antigen that is xenogeneic to a Her2/neu antigen expressed by mammary gland cells of a dog for use in the treatment of canine mammary gland tumor in a dog suffering from canine mammary gland carcinoma/tumor, wherein the xenogeneic mammary gland carcinoma/tumor-associated Her2/neu antigen is a vector comprising a DNA sequence encoding the xenogeneic therapeutic mammary gland carcinoma/tumor-associated Her2/neu antigen under the control of a promoter which promotes expression of the xenogeneic mammary gland tumor-associated Her2/neu antigen in the dog, and wherein the vector has the sequence comprising 106-3885 of the sequence as set forth in SEQ ID NO:1 and the xenogeneic Her2/neu antigen is administered post-surgical removal of tumors in subjects suffering from said cancers.

4. The xenogeneic Her2/neu antigen according to claim 1 or 2 for use according to claim 1 or 2, wherein:

   1) the Her2/neu-associated carcinoma is surgically debulked;
   2) a prime immunization comprising a first plasmid encoding a xenogeneic Her2/neu antigen is administered; and
   3) a booster immunization is administered viaelectrotransfer/electroporation; wherein the booster is either the first plasmid, or is a second plasmid capable of expressing in vivo in a canine a different xenogeneic Her2/neu antigen, including those encoded by SEQ ID NO:3 or 4, or is a recombinant vector capable of expressing in vivo any Her2/neu protein, which is capable of eliciting a therapeutically effective immune response against heterologous Her2/neu expressed by the Her2/neu associated carcinoma.

5. The xenogeneic Her2/neu antigen according to claim 4 for use according to claim 4, wherein:

   1) the prime immunization is administered without a needle;
   2) the first plasmid is capable of expressing in vivo in a canine a sequence as set forth in SEQ ID NO:2;
   3) the booster immunization comprises administration of the plasmid of step 2.

6. The xenogeneic Her2/neu antigen according to claim 4 or 5 for use according to claim 4 or 5, wherein the booster immunization is provided to surviving canines once every 3 to 6 months.

7. The xenogeneic Her2/neu antigen according to any one of claim 1 or 2 for use according to claim 1 or 2, wherein the xenogeneic differentiation antigen is administered by DNA immunization of the subject with DNA encoding the xenogeneic differentiation antigen in a non-viral plasmid vector comprising DNA encoding the xenogeneic differentiation antigen under the control of a promoter which promotes expression of the xenogeneic differentiation antigen.

8. The xenogeneic Her2/neu antigen according to any one of claims 1 or 2 for use according to any one of claims 1, 2 or 7 performed concurrently with resection of a mammary gland tumor (MGT).

9. A vector that is capable of expressing in vivo in a canine the protein as set forth in SEQ ID NO:2 for use in the treatment of canine mammary carcinoma/tumors in a dog suffering from canine mammary carcinoma/tumor, wherein the canine mammary carcinoma/tumor is a Her2/neu associated mammary carcinoma/tumor, wherein the vector is administered post-surgical removal of tumors in subjects suffering from said cancers.

10. Use of a xenogeneic Her2/neu antigen that is xenogeneic to a Her2/neu antigen expressed by mammary cells of a dog for the manufacture of a medicament for the treatment of canine mammary carcinoma/tumor in a dog suffering from canine mammary carcinoma/tumor, wherein the xenogeneic Her2/neu antigen is in an immunological effective amount and the xenogeneic Her2/neu antigen is administered post-surgical removal of tumors in subjects suffering

from said cancers, and wherein the xenogenic mammary gland tumor-associated Her2/neu antigen is a vector comprising a DNA sequence encoding the xenogeneic therapeutic mammary gland tumor-associated Her2/neu antigen under the control of a promoter which promotes expression of the mammary gland tumor-associated Her2/neu antigen in the dog.

11. Use of a xenogeneic Her2/neu antigen that is xenogeneic to a Her2/neu antigen expressed by mammary gland cells of a dog for the manufacture of a medicament for the treatment of canine mammary gland tumor in a dog suffering from canine mammary gland carcinoma/tumor, wherein the xenogeneic mammary gland carcinoma/tumor-associated Her2/neu antigen is a vector comprising a DNA sequence encoding the xenogeneic therapeutic mammary gland carcinoma/tumor-associated Her2/neu antigen under the control of a promoter which promotes expression of the xenogeneic mammary gland tumor associated Her2/neu antigen in the dog, and wherein the vector has the sequence comprising 106-3885 of the sequence as set forth in SEQ ID NO:1 and the xenogeneic Her2/neu antigen is administered post-surgical removal of tumors in subjects suffering from said cancers.

Patentansprüche

1. Xenogenes Her2/neu-Antigen, das gegenüber einem Her2/neu-Antigen, das von Brustdrüsenzellen eines Hundes exprimiert wird, xenogen ist, zur Verwendung bei der Behandlung eines caninen Mammakarzinoms/Mammatumors bei einem Hund, der an einem caninen Mammakarzinom/Mammatumor leidet, wobei das xenogene Her2/neu-Antigen in einer immunologisch wirksamen Menge vorliegt und wobei das xenogene Her2/neu-Antigen nach der chirurgischen Entfernung von Tumoren von Individuen, die an diesen Krebsarten leiden, verabreicht wird, und wobei das xenogene Her2/neu-Antigen, das mit Brustdrüsentumoren in Zusammenhang steht, ein Vektor ist, der eine DNA-Sequenz umfasst, die das xenogene therapeutische Her2/neu-Antigen, das mit Brustdrüsentumoren in Zusammenhang steht, unter der Kontrolle eines Promotors codiert, der die Expression des Her2/neu-Antigens, das mit Brustdrüsentumoren in Zusammenhang steht, in dem Hund fördert.

2. Xenogenes Her2/neu-Antigen nach Anspruch 1 zur Verwendung nach Anspruch 1, wobei das xenogene Differenzierungsantigen, das mit Mammatumoren in Zusammenhang steht, Her2/neu der Ratte ist.

3. Xenogenes Her2/neu-Antigen, das gegenüber einem Her2/neu-Antigen, das von Brustdrüsenzellen eines Hundes exprimiert wird, xenogen ist, zur Verwendung bei der Behandlung eines caninen Brustdrüsentumors bei einem Hund, der an einem caninen Brustdrüsenkarziom/Brustdrüsentumor leidet, wobei das xenogene Her2/neu-Antigen, das mit einem Brustdrüsenkarzinom/Brustdrüsentumor in Zusammenhang steht, ein Vektor ist, der eine DNA-Sequenz umfasst, die das xenogene therapeutische Her2/neu-Antigen, das mit einem Brustdrüsenkarzinom/Brustdrüsentumor in Zusammenhang steht, unter der Kontrolle eines Promotors codiert, der die Expression des xenogenen Her2/neu-Antigens, das mit Brustdrüsentumoren in Zusammenhang steht, in dem Hund fördert, und wobei der Vektor die Sequenz aufweist, die die Positionen 106 bis 3885 der in SEQ ID NO:1 gezeigten Sequenz umfasst, und wobei das xenogene Her2/neu-Antigen nach der chirurgischen Entfernung von Tumoren von Individuen, die an diesen Krebsarten leiden, verabreicht wird.

4. Xenogenes Her2/neu-Antigen nach Anspruch 1 oder 2 zur Verwendung nach Anspruch 1 oder 2, wobei:

1) das Karzinom, das mit Her2/neu im Zusammenhang steht, chirurgisch teilweise entfernt wird (debulked);
2) eine Prime-Immunisierung, die ein erstes Plasmid umfasst, das ein xenogenes Her2/neu-Antigen codiert, verabreicht wird; und
3) eine Booster-Immunisierung über Elektrotransfer/Elektroporation verabreicht wird;
wobei der Booster entweder das erste Plasmid oder ein zweites Plasmid ist, das in der Lage ist, *in vivo* in einem Individuum der Canidae ein anderes xenogenes Her2/neu-Antigen zu exprimieren, einschließlich derjenigen, die durch SEQ ID NO:3 oder 4 codiert werden, oder wobei der Booster ein rekombinanter Vektor ist, der in der Lage ist, *in vivo* ein beliebiges Her2/neu-Protein zu exprimieren, das in der Lage ist, eine therapeutisch wirksame Immunantwort gegen ein heterologes Her2/neu auszulösen, das von dem Karzinom, das mit Her2/neu in Zusammenhang steht, exprimiert wird.

5. Xenogenes Her2/neu-Antigen nach Anspruch 4 zur Verwendung nach Anspruch 4, wobei:

1) die Prime-Immunisierung ohne eine Nadel verabreicht wird;
2) das erste Plasmid in der Lage ist, *in vivo* in einem Individuum der Canidae eine wie in SEQ ID NO:2 gezeigte

Sequenz zu exprimieren;
3) die Booster-Immunisierung die Verabreichung des Plasmids nach Schritt 2 umfasst.

6. Xenogenes Her2/neu-Antigen nach Anspruch 4 oder 5 zur Verwendung nach Anspruch 4 oder 5, wobei die Booster-Immunisierung überlebenden Individuen der Canidae einmal alle 3 bis 6 Monate versehen wird.

7. Xenogenes Her2/neu-Antigen nach einem der Ansprüche 1 oder 2 zur Verwendung nach Anspruch 1 oder 2, wobei das xenogene Differenzierungsantigen durch DNA-Immunisierung des Individuums mit DNA, die das xenogene Differenzierungsantigen codiert, in einem nicht-viralen Plasmidvektor verabreicht wird, der DNA umfasst, die das xenogene Differenzierungsantigen unter der Kontrolle eines Promotors codiert, der die Expression des xenogenen Differenzierungsantigens fördert.

8. Xenogenes Her2/neu-Antigen nach einem der Ansprüche 1 oder 2 zur Verwendung nach einem der Ansprüche 1, 2 oder 7, die gleichzeitig mit der Resektion eines Brustdrüsentumors (mammary gland tumor; MGT) durchgeführt wird.

9. Vektor, der in der Lage ist, *in vivo* in einem Individuum der Canidae das wie in SEQ ID NO:2 gezeigte Protein zu exprimieren, zur Verwendung bei der Behandlung eines caninen Mammakarzinoms/Mammatumors bei einem Hund, der an einem caninen Mammakarzinom/Mammatumor leidet, wobei das canine Mammakarzinom/der canine Mammatumor ein Mammakarzinom/Mammatumor ist, das/der mit Her2/neu in Zusammenhang steht, wobei der Vektor nach der chirurgischen Entfernung von Tumoren von Individuen, die an diesen Krebsarten leiden, verabreicht wird.

10. Verwendung eines xenogenen Her2/neu-Antigens, das xenogen gegenüber einem Her2/neu-Antigen ist, das von den Brustdrüsenzellen eines Hundes exprimiert wird, zur Herstellung eines Medikaments zur Behandlung eines caninen Mammakarzinoms/Mammatumors bei einem Hund, der an einem caninen Mammakarzinom/Mammatumor leidet, wobei das xenogene Her2/neu-Antigen in einer immunologisch wirksamen Menge vorliegt und das xenogene Her2/neu-Antigen nach der chirurgischen Entfernung von Tumoren von Individuen, die an diesen Krebsarten leiden, verabreicht wird, und wobei das xenogene Her2/neu-Antigen, das mit einem Brustdrüsentumor in Zusammenhang steht, ein Vektor ist, der eine DNA-Sequenz umfasst, die das xenogene therapeutische Her2/neu-Antigen, das mit einem Brustdrüsentumor in Zusammenhang steht, unter der Kontrolle eines Promotors codiert, der die Expression des Her2/neu-Antigens, das mit einem Brustdrüsentumor in Zusammenhang steht, in dem Hund fördert.

11. Verwendung eines xenogenen Her2/neu-Antigens, das gegenüber einem Her2/neu-Antigen xenogen ist, das von den Brustdrüsenzellen eines Hundes exprimiert wird, zur Herstellung eines Medikaments zur Behandlung eines caninen Brustdrüsentumors bei einem Hund, der an einem caninen Brustdrüsenkarzinom/Brustdrüsentumor leidet, wobei das xenogene Her2/neu-Antigen, das mit einem Brustdrüsenkarzinom/Brustdrüsentumor in Zusammenhang steht, ein Vektor ist, der eine DNA-Sequenz umfasst, die das xenogene therapeutische Her2/neu-Antigen, das mit einem Brustdrüsenkarzinom/Brustdrüsentumor in Zusammenhang steht, unter der Kontrolle eines Promotors codiert, der die Expression des xenogenen Her2/neu-Antigens, das mit einem Brustdrüsentumor in Zusammenhang steht, in dem Hund fördert, und wobei der Vektor die Sequenz aufweist, die die Positionen 106 bis 3885 der in SEQ ID NO:1 gezeigten Sequenz umfasst, und wobei das xenogene Her2/neu-Antigen nach der chirurgischen Entfernung von Tumoren von Individuen, die an diesen Krebsarten leiden, verabreicht wird.

Revendications

1. Antigène Her2/neu xénogénique qui est xénogénique par rapport à un antigène Her2/neu exprimé par des cellules mammaires d'un chien pour une utilisation dans le traitement d'un carcinome/d'une tumeur mammaire canin(e) chez un chien souffrant d'un carcinome/d'une tumeur mammaire canin(e), l'antigène Her2/neu xénogénique étant dans une quantité immunologique efficace et l'antigène Her2/neu xénogénique étant administré après l'élimination chirurgicale des tumeurs chez les sujets souffrant desdits cancers, et l'antigène Her2/neu xénogénique associé à une tumeur de la glande mammaire étant un vecteur comprenant une séquence d'ADN codant pour l'antigène Her2/neu xénogénique associé à une tumeur de la glande mammaire thérapeutique sous le contrôle d'un promoteur qui favorise l'expression de l'antigène Her2/neu associé à une tumeur de la glande mammaire chez le chien.

2. Antigène Her2/neu xénogénique selon la revendication 1 pour une utilisation selon la revendication 1, l'antigène de différenciation xénogénique associé à une tumeur de la glande mammaire étant un Her2/neu de rat.

3. Antigène Her2/neu xénogénique qui est xénogénique par rapport à un antigène Her2/neu exprimé par des cellules de la glande mammaire d'un chien dans le traitement d'une tumeur de la glande mammaire canine chez un chien souffrant d'un carcinome/d'une tumeur de la glande mammaire canin(e), l'antigène Her2/neu xénogénique associé à un carcinome/une tumeur de la glande mammaire étant un vecteur comprenant une séquence d'ADN codant pour l'antigène Her2/neu xénogénique associé à un carcinome/une tumeur de la glande mammaire thérapeutique sous le contrôle d'un promoteur qui favorise l'expression de l'antigène Her2/neu xénogénique associé à une tumeur de la glande mammaire chez le chien, et le vecteur ayant la séquence comprenant 106 à 3885 de la séquence représentée par SEQ ID NO : 1 et l'antigène Her2/neu xénogénique étant administré après l'élimination chirurgicale des tumeurs chez des sujets souffrant desdits cancers.

4. Antigène Her2/neu xénogénique selon la revendication 1 ou la revendication 2 pour une utilisation selon la revendication 1 ou la revendication 2, où :

1) le carcinome associé à Her2/neu est réduit chirurgicalement ;
2) une immunisation de sensibilisation comprenant un premier plasmide codant pour un antigène Her2/neu xénogénique est administrée ; et
3) une immunisation de rappel est administrée par électrotransfert/électroporation ; le rappel soit étant le premier plasmide soit étant un second plasmide capable d'exprimer *in vivo* chez un canidé un antigène Her2/neu xénogénique différent, y compris ceux codés par SEQ ID NO : 3 ou 4, soit étant un vecteur recombinant capable d'exprimer *in vivo* toute protéine Her2/neu, qui est capable de déclencher une réponse immunitaire thérapeutiquement efficace contre un Her2/neu hétérologue exprimé par le carcinome associé à Her2/neu.

5. Antigène Her2/neu xénogénique selon la revendication 4 pour une utilisation selon la revendication 4, où :

1) l'immunisation de sensibilisation est administrée sans aiguille ;
2) le premier plasmide est capable d'exprimer *in vivo* chez un canidé une séquence telle que représentée par SEQ ID NO : 2 ;
3) l'immunisation de rappel comprend l'administration du plasmide de l'étape 2.

6. Antigène Her2/neu xénogénique selon la revendication 4 ou la revendication 5 pour une utilisation selon la revendication 4 ou la revendication 5, où l'immunisation de rappel est fournie à des canidés survivants une fois tous les 3 à 6 mois.

7. Antigène Her2/neu xénogénique selon l'une quelconque de la revendication 1 ou 2 pour une utilisation selon la revendication 1 ou 2, l'antigène de différenciation xénogénique étant administré par immunisation à base d'ADN du sujet avec l'ADN codant pour l'antigène de différenciation xénogénique dans un vecteur plasmidique non viral comprenant l'ADN codant pour l'antigène de différenciation xénogénique sous le contrôle d'un promoteur qui favorise l'expression de l'antigène de différenciation xénogénique.

8. Antigène Her2/neu xénogénique selon l'une quelconque des revendications 1 ou 2 pour une utilisation selon l'une quelconque des revendications 1, 2 ou 7 effectuée simultanément avec la résection de la tumeur de la glande mammaire (TGM).

9. Vecteur qui est capable d'exprimer *in vivo* chez un canidé la protéine telle que représentée par SEQ ID NO : 2 pour une utilisation dans le traitement de carcinomes/tumeurs mammaires canin(e)s chez un chien souffrant d'un carcinome/d'une tumeur mammaire canin(e), le carcinome/la tumeur mammaire canin(e) étant un carcinome/une tumeur mammaire associé(e) à Her2/neu, le vecteur étant administré après l'élimination chirurgicale des tumeurs chez des sujets souffrant desdits cancers.

10. Utilisation d'un antigène Her2/neu xénogénique qui est xénogénique par rapport à un antigène Her2/neu exprimé par des cellules mammaires d'un chien pour la fabrication d'un médicament destiné au traitement d'un carcinome/d'une tumeur mammaire canin(e) chez un chien souffrant d'un carcinome/d'une tumeur mammaire canin(e), l'antigène Her2/neu xénogénique étant dans une quantité immunologique efficace et l'antigène Her2/neu xénogénique étant administré après l'élimination chirurgicale des tumeurs chez des sujets souffrant desdits cancers, et l'antigène Her2/neu xénogénique associé à une tumeur de la glande mammaire étant un vecteur comprenant une séquence d'ADN codant pour l'antigène Her2/neu xénogénique associé à une tumeur de la glande mammaire thérapeutique sous le contrôle d'un promoteur qui favorise l'expression de l'antigène Her2/neu associé à une tumeur de la glande mammaire chez le chien.

11. Utilisation d'un antigène Her2/neu xénogénique qui est xénogénique par rapport à un antigène Her2/neu exprimé par des cellules des glandes mammaires d'un chien pour la production d'un médicament destiné au traitement d'une tumeur de la glande mammaire canine chez un chien souffrant d'un carcinome/d'une tumeur de la glande mammaire canin(e), l'antigène Her2/neu xénogénique associé à un carcinome/une tumeur de la glande mammaire étant un vecteur comprenant une séquence d'ADN codant pour l'antigène Her2/neu xénogénique associé à un carcinome/une tumeur de la glande mammaire thérapeutique sous le contrôle d'un promoteur qui favorise l'expression de l'antigène Her2/neu xénogénique associé à une tumeur de la glande mammaire chez le chien, et le vecteur ayant la séquence comprenant 106 à 3885 de la séquence telle que représentée par SEQ ID NO : 1 et l'antigène Her2/neu xénogénique étant administré après l'élimination chirurgicale des tumeurs chez des sujets souffrant desdits cancers.

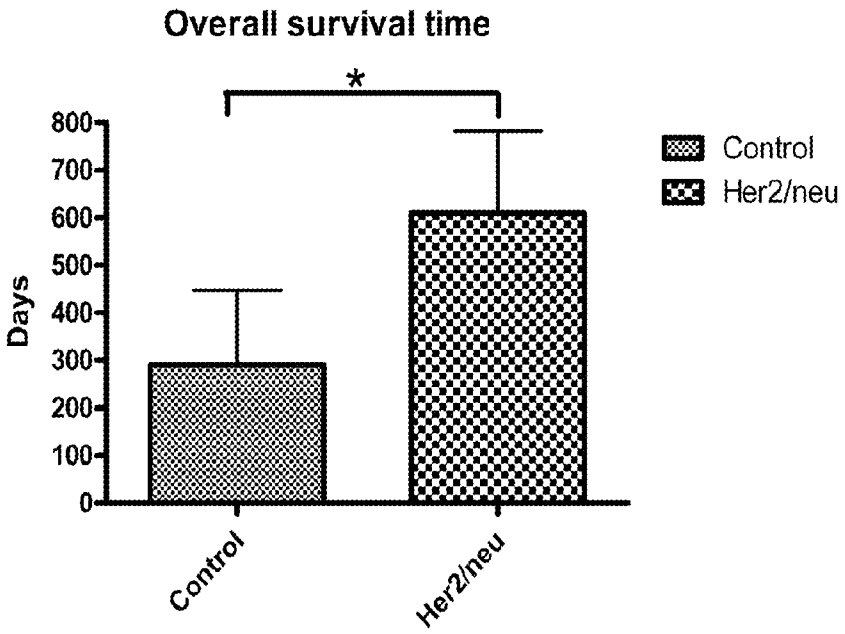
Overall survival time
*
800
700
600
500
400
300
200
100
0
Days
Control
Her2/neu
Control
Her2/neu

FIG. 1A

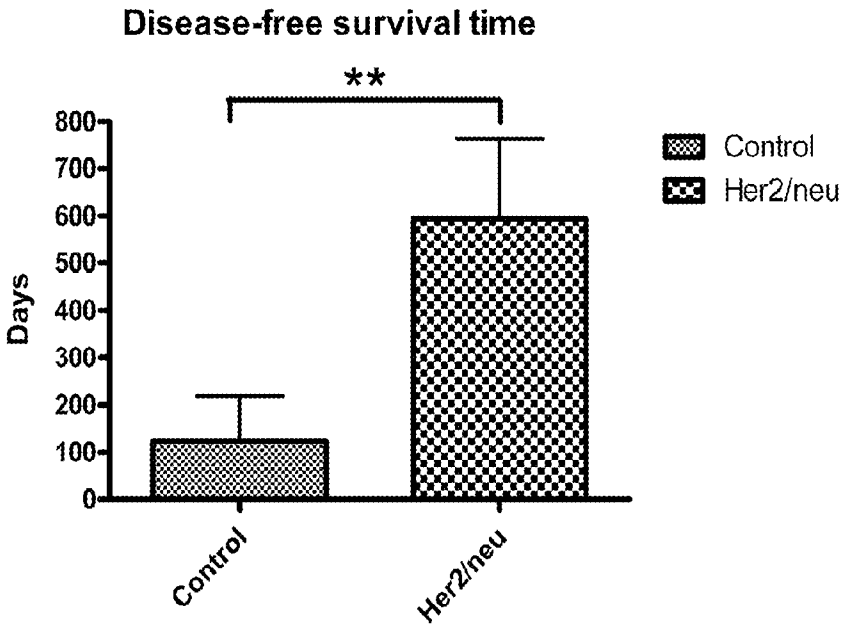
Disease-free survival time
**
800
700
600
500
400
300
200
100
0
Days
Control
Her2/neu
Control
Her2/neu

FIG. 1B

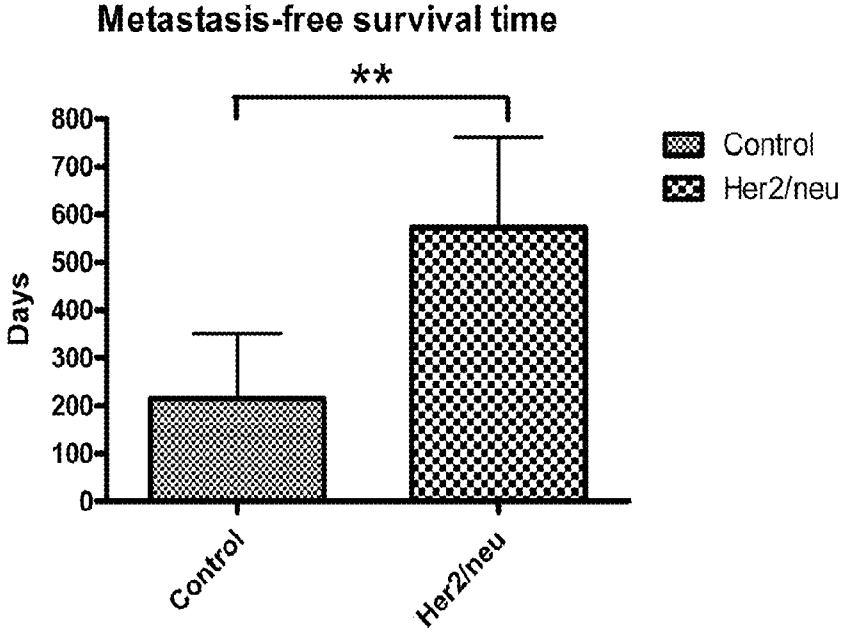
Metastasis-free survival time
**
800
700
600
500
400
300
200
100
0
Days
Control
Her2/neu
Control
Her2/neu

FIG. 1C

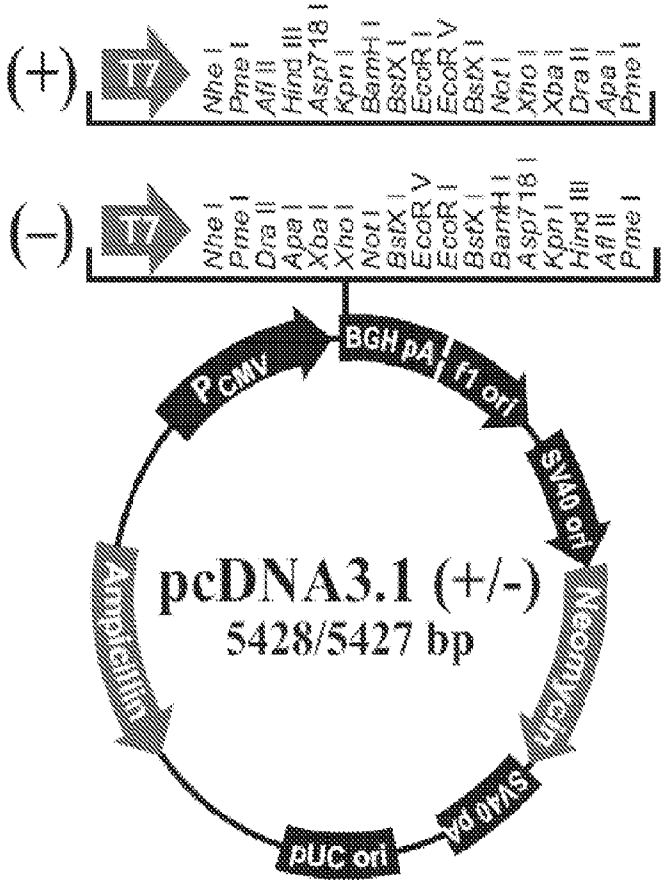
(+)
T7
Nhe I
Pme I
Afl II
Hind III
Asp718 I
Kpn I
BamH I
BstX I
EcoR I
EcoR V
BstX I
Not I
Xho I
Xba I
Dra III
Apa I
Pme I
(−)
T7
Nhe I
Pme I
Dra III
Apa I
Xba I
Xho I
Not I
BstX I
EcoR V
EcoR I
BstX I
BamH I
Asp718 I
Kpn I
Hind III
Afl II
Pme I
P CMV
BGH pA
f1 ori
SV40 ori
Neomycin
SV40 pA
pUC ori
Ampicillin
pcDNA3.1 (+/-)
5428/5427 bp

FIG. 2

```
   1 ttggctattg gccattgcat acgttgtatc tatatcataa tatgtacatt
  51 tatattggct catgtccaat atgaccgcca tgttgacatt gattattgac
 101 tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata
 151 tggagttccg cgttacataa cttacggtaa atggcccgcc tggctgaccg
 201 cccaacgacc cccgcccatt gacgtcaatg atgacgtatg ttcccatagt
 251 aacgccaata gggactttcc attgacgtca atgggtggag tatttacggt
 301 aaactgccca cttggcagta catcaagtgt atcatatgcc aagtccgccc
 351 cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta
 401 catgacctta cgggactttc ctacttggca gtacatctac gtattagtca
 451 tcgctattac catggtgatg cggttttggc agtacaccaa tgggcgtgga
 501 tagcggtttg actcacgggg atttccaagt ctccacccca ttgacgtcaa
 551 tgggagtttg ttttggcacc aaaatcaacg ggactttcca aaatgtcgta
 601 ataaccccgc cccgttgacg caaatgggcg gtaggcgtgt acggtgggag
 651 gtctatataa gcagagctcg tttagtgaac cgtcagatcg cctggagacg
 701 ccatccacgc tgttttgacc tccatagaag acaccgggac cgatccagcc
 751 tccgcggccg ggaacggtgc attggaacgc ggattccccg tgccaagagt
 801 gacgtaagta ccgcctatag actctatagg cacacccctt tggctcttat
 851 gcatgctata ctgtttttgg cttggggcct atacaccccc gcttccttat
 901 gctataggtg atggtatagc ttagcctata ggtgtgggtt attgaccatt
 951 attgaccact cccctattgg tgacgatact ttccattact aatccataac
1001 atggctcttt gccacaacta tctctattgg ctatatgcca atactctgtc
1051 cttcagagac tgacacggac tctgtatttt tacaggatgg ggtcccattt
1101 attatttaca aattcacata tacaacaacg ccgtcccccg tgcccgcagt
1151 ttttattaaa catagcgtgg gatctccacg cgaatctcgg gtacgtgttc
1201 cggacatggg ctcttctccg gtagcggcgg agcttccaca tccgagccct
1251 ggtcccatgc ctccagcggc tcatggtcgc tcggcagctc cttgctccta
1301 acagtggagg ccagacttag gcacagcaca atgcccacca ccaccagtgt
1351 gccgcacaag gccgtggcgg tagggtatgt gtctgaaaat gagctcggag
1401 attgggctcg caccgctgac gcagatggaa gacttaaggc agcggcagaa
1451 gaagatgcag gcagctgagt tgttgtattc tgataagagt cagaggtaac
1501 tcccgttgcg gtgctgttaa cggtggaggg cagtgtagtc tgagcagtac
1551 tcgttgctgc cgcgcgcgcc accagacata atagctgaca gactaacaga
               NcoI (1611)   PstI (1624)
1601 ctgttccttt ccatgggtct tttctgcagt caccgtccac gcgttaatac
1651 gactcactat agggagaccc aagctggcta gcgtttaaac ttaagcttgg
               BamHI (1711)             EcoRI (1734)
1701 taccgagctc ggatccacta gtccagtgtg gtggaattcc gggaaga
```

FIG. 3 (1/4)

```
3338 aaggcttagg caatagagta gggccaaaaa gcctgacctc actctaactc
3388 aaagtaatgt ccaggttccc agagaatatc tgctggtatt tttctgtaaa
3438 gaccatttgc aaaattgtaa cctaatacaa agtgtagcct tcttccaact
3488 caggtagaac acacctgtct ttgtcttgct gttttcactc agcccttta
3538 acattttccc ctaagcccat atgtctaagg aaaggatgct atttggtaat
3588 gaggaactgt tatttgtatg tgaattaaag tgctcttatt ttaaaaaacc
3638 ggaattctgc agatatccag cacagtggcg gccgctcgag tctagagggc
3688 ccgtttaaac ccgctgatca gcctcgactg tgccttctag ttgccagcca
3738 tctgttgttt gcccctcccc cgtgccttcc ttgaccctgg aaggtgccac
3788 tcccactgtc ctttcctaat aaaatgagga attgcatcg cattgtctga
3838 gtaggtgtca ttctattctg gggggtgggg tggggcagga cagcaagggg
3888 gaggattggg aagacaatag caggcatggt ggggatgcag gggggggggg
3938 gcgctgaggt ctgcctcgtg aagaaggtgt tgctgactca taccaggcct
3988 gaatcgcccc atcatccagc cagaaagtga gggagccacg gttgatgaga
4038 gctttgttgt aggtggacca gttggtgatt ttgaactttt gctttgccac
4088 ggaacggtct gcgttgtcgg gaagatgcgt gatctgatcc ttcaactcag
4138 caaaagttcg atttattcaa caaagccgcc gtcccgtcaa gtcagcgtaa
4188 tgctctgcca gtgttacaac caattaacca attctgatta gaaaaactca
4238 tcgagcatca aatgaaactg caatttattc atatcaggat tatcaatacc
4288 atatttttga aaaagccgtt tctgtaatga aggagaaaac tcaccgaggc
4338 agttccatag gatggcaaga tcctggtatc ggtctgcgat tccgactcgt
4388 ccaacatcaa tacaacctat taatttcccc tcgtcaaaaa taaggttatc
4438 aagtgagaaa tcaccatgag tgacgactga atccggtgag aatggcaaaa
4488 gcttatgcat ttctttccag acttgttcaa caggccagcc attacgctcg
4538 tcatcaaaat cactcgcatc aaccaaaccg ttattcattc gtgattgcgc
4588 ctgagcgaga cgaaatacgc gatcgctgtt aaaaggacaa ttacaaacag
4638 gaatcgaatg caaccggcgc aggaacactg ccagcgcatc aacaatattt
4688 tcacctgaat caggatattc ttctaatacc tggaatgctg ttttcccggg
4738 gatcgcagtg gtgagtaacc atgcatcatc aggagtacgg ataaaatgct
4788 tgatggtcgg aagaggcata aattccgtca gccagtttag tctgaccatc
4838 tcatctgtaa catcattggc aacgctacct ttgccatgtt tcagaaacaa
4888 ctctggcgca tcgggcttcc catacaatcg atagattgtc gcacctgatt
4938 gcccgacatt atcgcgagcc catttatacc catataaatc agcatccatg
                      XhoI (5006)
4988 ttggaattta atcgcggcct cgagcaagac gtttcccgtt gaatatggct
5038 cataacaccc cttgtattac tgtttatgta agcagacagt tttattgttc
5088 atgatgatat atttttatct tgtgcaatgt aacatcagag attttgagac
                               PstI (5162)
5138 acaacgtggc tttcccccc ccccctgcag cgtttcttcc ttttccccac
```

FIG. 3 (2/4)

```
5188 cccacccccc aagttcgggt gaaggcccag ggctcgcagc caacgtcggg
5238 gcggcaggcc ctgccatagc ctcaggttac tcatatatac tttagattga
5288 tttaaaactt cattttaat ttaaaaggat ctaggtgaag atccttttg
5338 ataatctcat gaccaaaatc ccttaacgtg agttttcgtt ccactgagcg
5388 tcagaccccg tagaaaagat caaaggatct tcttgagatc ctttttttct
5438 gcgcgtaatc tgctgcttgc aaacaaaaaa accaccgcta ccagcggtgg
5488 tttgtttgcc ggatcaagag ctaccaactc tttttccgaa ggtaactggc
5538 ttcagcagag cgcagatacc aaatactgtt cttctagtgt agccgtagtt
5588 aggccaccac ttcaagaact ctgtagcacc gcctacatac ctcgctctgc
5638 taatcctgtt accagtggct gctgccagtg gcgataagtc gtgtcttacc
5688 gggttggact caagacgata gttaccggat aaggcgcagc ggtcgggctg
5738 aacgggggt tcgtgcacac agcccagctt ggagcgaacg acctacaccg
5788 aactgagata cctacagcgt gagctatgag aaagcgccac gcttcccgaa
5838 gggagaaagg cggacaggta tccggtaagc ggcagggtcg gaacaggaga
5888 gcgcacgagg gagcttccag ggggaaacgc ctggtatctt tatagtcctg
5938 tcgggtttcg ccacctctga cttgagcgtc gatttttgtg atgctcgtca
5988 gggggggcgga gcctatggaa aaacgccagc aacgcggcct ttttacggtt
6038 cctggccttt tgctggcctt ttgctcacat gttctttcct gcgttatccc
6088 ctgattctgt ggataaccgt attaccgcca tgcattagtt attaatagta
6138 atcaattacg gggtcattag ttcatagccc atatatggag ttccgcgtta
                    BglI (6206)
6188 cataacttac ggtaaatggc ccgcctggct gaccgcccaa cgacccccgc
6238 ccattgacgt caataatgac gagatctgat ataggtgaca gacgatatga
6288 ggctatatcg ccgatagagg cgacatcaag ctggcacatg gccaatgcat
6338 atcgatctat acattgaatc aatattggca attagccata ttagtcattg
6388 gttatatagc ataaatcaat a
```

FIG. 3 (3/4)

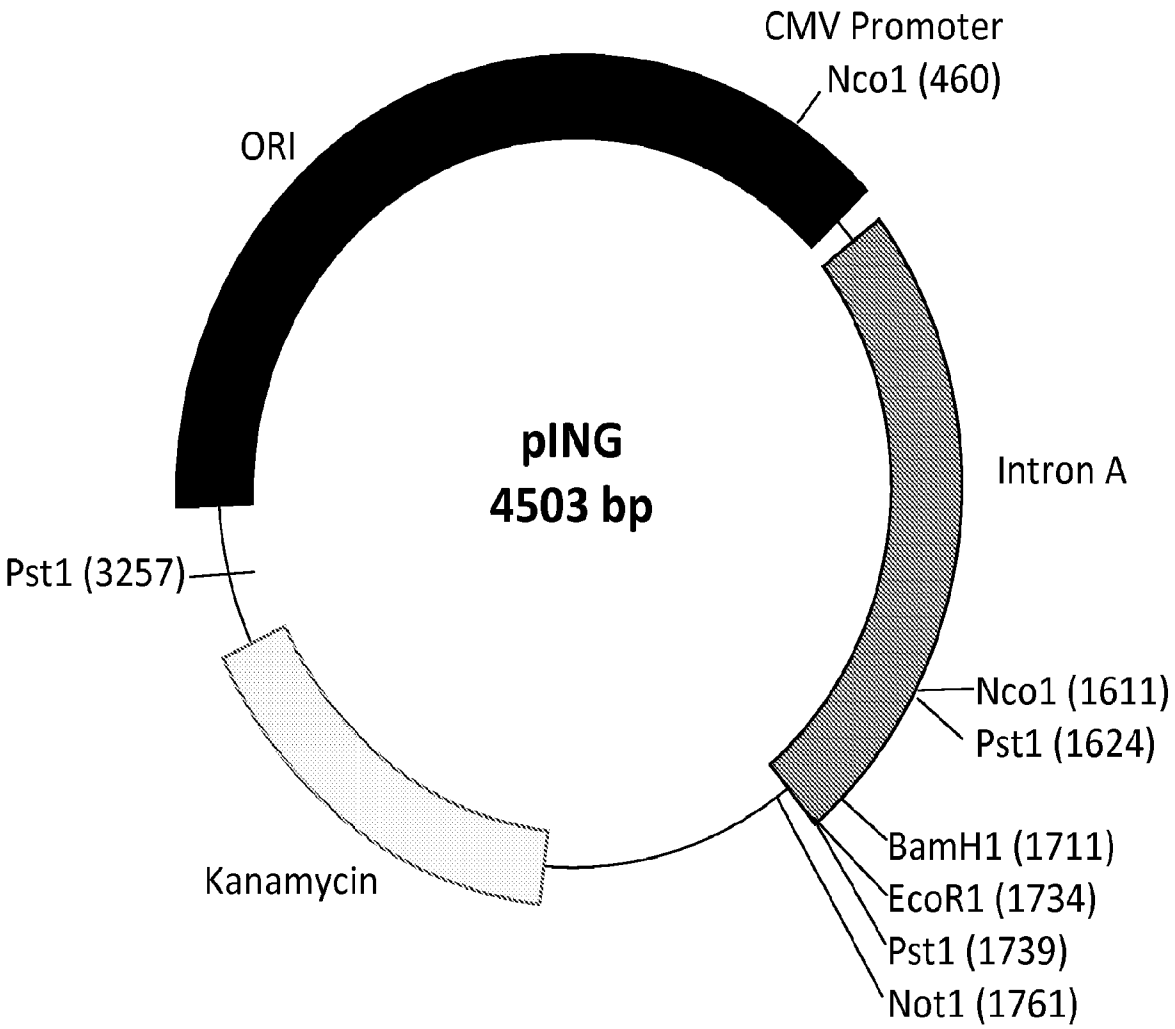
CMV Promoter
Nco1 (460)
ORI
pING
4503 bp
Intron A
Pst1 (3257)
Nco1 (1611)
Pst1 (1624)
BamH1 (1711)
EcoR1 (1734)
Pst1 (1739)
Not1 (1761)
Kanamycin

FIG. 3 (4/4)

REFERENCES CITED IN THE DESCRIPTION

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1. Xenogén Her2/neu antigén, amely xenogén kutya emlősejtjei által expresszált Her2/neu antigénhez képest, kutyafélék emlőkarcinómája/daganata kezelésében történő alkalmazásra kutyafélék emlőkarcinómájában/daganatában szenvedő kutyában, ahol a xenogén Her2/neu antigén immunológiailag hatásos mennyiségben van és a xenogén Her2/neu antigén daganatok műtéti eltávolítása után van beadva a rákoktól szenvedő alanyoknak, és ahol a xenogén emlőmirigy daganattal kapcsolatos Her2/neu antigén olyan vektor, amely a xenogén terápiás emlőmirigy daganattal kapcsolatos Her2/neu antigént kódoló DNS-szekvenciát tartalmaz olyan promóter szabályzása alatt, amely elősegíti az emlőmirigy daganattal kapcsolatos Her2/neu antigén expresszióját a kutyában.

2. Az 1. igénypont szerinti xenogén Her2/neu antigén az 1. igénypont szerinti alkalmazásra, ahol a xenogén emlőmirigy daganattal kapcsolatos antigén patkány Her2/neu.

3. Xenogén Her2/neu antigén, amely xenogén kutya emlőmirigy sejtjei által expresszált Her2/neu antigénhez képest, kutyafélék emlőmirigy daganata kezelésében történő alkalmazásra kutyafélék emlőmirigy karcinómájában/daganatában szenvedő kutyában, ahol a xenogén emlőmirigy karcinómával/daganattal kapcsolatos Her2/neu antigén olyan vektor, amely a xenogén terápiás emlőmirigy karcinómával/daganattal kapcsolatos Her2/neu antigént kódoló DNS-szekvenciát tartalmaz olyan promóter szabályzása alatt, amely elősegíti az emlőmirigy daganattal kapcsolatos Her2/neu antigén expresszióját a kutyában, és ahol a vektor szekvenciája a SEQ ID NO:1 szerinti szekvencia 106-3885 szekvenciáját tartalmazza és a xenogén Her2/neu antigén daganatok műtéti eltávolítása után van beadva a rákoktól szenvedő alanoknak.

4. Az 1. vagy 2. igénypont szerinti xenogén Her2/neu antigén az 1. vagy 2. igénypont szerinti alkalmazásra, ahol:

1) a Her2/neu-val kapcsolatos karcinóma műtétileg kisebbítve van;

2) egy első, xenogén Her2/neu antigént kódoló plazmidot tartalmazó beindító immunizálás van adva; és

3) emlékeztető immunizálás van adva elektrotranszfer/elektroporáció útján;

ahol az emlékeztető vagy az első plazmid, vagy egy második plazmid, amely képes in vivo expresszálni kutyafélékben egy eltérő xenogén Her2/neu antigént, beleértve a SEQ ID NO:3 vagy 4 által kódoltakat, vagy rekombináns vektor, amely képes expresszálni in vivo bármilyen Her2/neu proteint, amely képes terápiásan hatásos immunválaszt kiváltani a Her2/neu-val kapcsolatos karcinóma által expresszált heterológ Her2/neu ellen.

5. A 4. igénypont szerinti xenogén Her2/neu antigén a 4. igénypont szerinti alkalmazásra, ahol:

1) az első immunizálás tű nélkül van adva;

2) az első plazmid képes in vivo expresszálni kutyafélékben a SEQ ID NO:2 szerinti szekvenciát;

3) az emlékeztető immunizálás a 2. lépésbeli plazmid beadását foglalja magában.

6. A 4. vagy 5. igénypont szerinti xenogén Her2/neu antigén a 4. vagy 5. igénypont szerinti alkalmazásra, ahol az emlékeztető immunizálás a túlélő kutyaféléknek 3 - 6 havonta van adva.

7. Az 1. vagy 2. igénypont szerinti xenogén Her2/neu antigén az 1. vagy 2. igénypont szerinti alkalmazásra, ahol a xenogén differenciációs antigén az alany DNS-immunizálásával van beadva a differenciációs antigént nem-virális plazmidvektorban kódoló DNS-sel, amely a xenogén differenciációs antigént kódoló DNS-t olyan promóter szabályozása alatt tartalmazza, amely elősegíti a xenogén differenciációs antigén expresszióját.

8. Az 1. vagy 2. igénypont szerinti xenogén Her2/neu antigén az 1., 2. vagy 7. igénypont szerinti alkalmazásra, az emlőmirigy daganat (MGT) kivágásával egyidejűleg végezve.

9. Vektor, amely képes in vivo kutyafélékben SEQ ID NO:2 szerinti proteint expresszálni, kutyafélék emlőkarcinómája/daganata kezelésében történő alkalmazásra kutyafélék emlőmirigy karcinómájában/daganatában szenvedő kutyában, ahol a kutyaféle emlőkarcinóma/daganat Her2/neu-val kapcsolatos emlőkarcinóma/daganat, ahol a vektor daganatok műtéti eltávolítása után van beadva a rákoktól szenvedő alanyoknak.

10. Xenogén Her2/neu antigén, amely xenogén kutya emlősejtjei által expresszált Her2/neu antigénhez képest, alkalmazása kutyafélék emlőkarcinómája/daganata kutyafélék emlőkarcinómájában/daganatában szenvedő kutyában való kezelésére szolgáló gyógyszer gyártására, ahol a xenogén Her2/neu antigén immunológiailag hatásos mennyiségben van és a xenogén Her2/neu antigén daganatok műtéti eltávolítása után van beadva a rákoktól szenvedő alanyoknak, és ahol a xenogén emlőmirigy daganattal kapcsolatos Her2/neu antigén olyan vektor, amely a xenogén terápiás emlőmirigy daganattal kapcsolatos Her2/neu antigént kódoló DNS-szekvenciát tartalmaz olyan promóter szabályzása alatt, amely elősegíti az emlőmirigy daganattal kapcsolatos Her2/neu antigén expresszióját a kutyában.

11. Xenogén Her2/neu antigén, amely xenogén kutya emlőmirigy sejtjei által expresszált Her2/neu antigénhez képest, alkalmazása kutyafélék emlőmirigy daganata kutyafélék emlőmirigy karcinómájában/daganatában szenvedő kutyában való kezelésére szolgáló gyógyszer gyártására, ahol a xenogén emlőmirigy karcinómával/daganattal kapcsolatos Her2/neu antigén olyan vektor, amely a xenogén terápiás emlőmirigy karcinómával/daganattal kapcsolatos Her2/neu antigént kódoló DNS-szekvenciát tartalmaz olyan promóter szabályzása alatt, amely elősegíti az emlőmirigy daganattal kapcsolatos Her2/neu antigén expresszióját a kutyában, és ahol a vektor szekvenciája a SEQ ID NO:1 szerinti szekvencia 106-3885 szekvenciáját tartalmazza és a xenogén Her2/neu antigén daganatok műtéti eltávolítása után van beadva a rákoktól szenvedő alanyoknak.